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TOTAL PHOSPHORUS CONTENT IN THE WATERS OF THE ARABIAN SEA ALONG THE WEST COAST OF INDIA

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(Communicated by Dr. N. K. Panikkar, F.N.I.)

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Total phosphorus estimations are made on samples collected along four sections, perpendicular to the coast between Bombay and Cochin, during the 25th cruise of INS *Kistna* in March 1965. The results reveal considerable variability in the regional and depthwise distribution of the same. Higher concentrations are encountered in the upper 800 m in the northern sections (off Bombay and Karwar) than in the southern ones (off Mangalore and Cochin) while at deeper levels (> 800 m) the trend is reverse. Off Cochin greater concentrations (> 10 μg-at/l) are found below 800 m as compared with 2–4 μg-at/l found at the same depths in the other regions. Percentage of organic phosphorus is in general very high in the surface (upper 200 m). Even at 1,000 m mineralization of organic phosphorus is not complete and at majority of the stations the composition amounted to 20–30 per cent of the total phosphorus. Distinct convergence of the waters is indicated almost all along the coast over the mid-shelf, extending towards the slope region. Probable factors governing the distribution of total phosphorus are discussed.

Introduction

The significance of the total phosphorus concentration in the sea as an index of potential fertility of waters and for identifying different water masses as tracer has been emphasized earlier (Redfield *et al.* 1937; Armstrong and Harvey 1950; Bush *et al.* 1955; Rochford 1958). While considerable information is available on inorganic phosphate content of the waters in the Arabian Sea, very little is known on the distribution of total phosphorus. Earlier studies on the total phosphorus of the west coast of India were made by Seshappa and Jayaraman (1956) and Rao (1957). The present account is based on the observations made in March 1965 during the 25th cruise of INS *Kistna* which was undertaken as part of the Indian Programme of the International Indian Ocean Expedition.

Methods

Location of the stations from where samples of water were collected for the analysis of nutrients has been indicated in Fig. 1. The four sections, perpendicular to the coast, run approximately (1) off Bombay, (2) off Karwar,

(3) off Mangalore and (4) off Cochin. Water samples were collected in clean heavy polythene bottles and were preserved by freezing at —10 °C for subsequent analysis at the shore laboratory.

Total phosphorus was estimated by the method described by Hansen and Robinson (1953), digesting the organic matter with perchloric acid. Inorganic phosphates were estimated by the method adopted by Wooster and Rakestraw (1951).

Results and Discussion

The distribution of total and inorganic phosphorus along the four sections has been represented in Figs. 2–7. Detailed discussion of inorganic phosphates

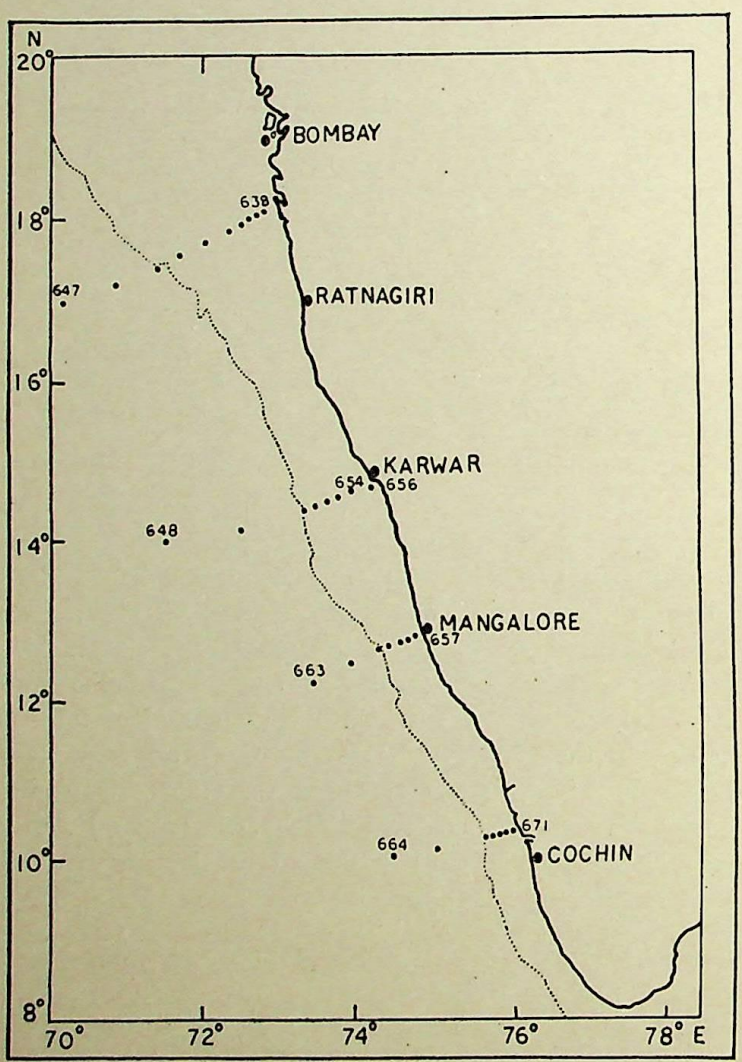

Fig. 1. Showing station locations.

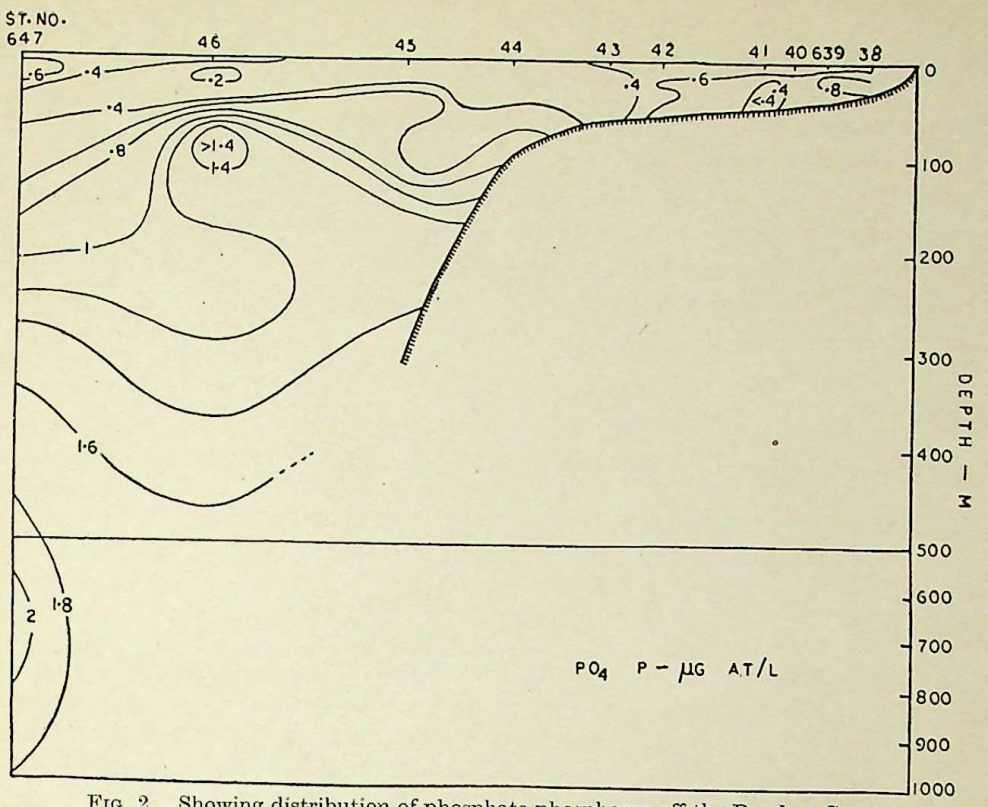

FIG. 2. Showing distribution of phosphate-phosphorus off the Bombay Coast.

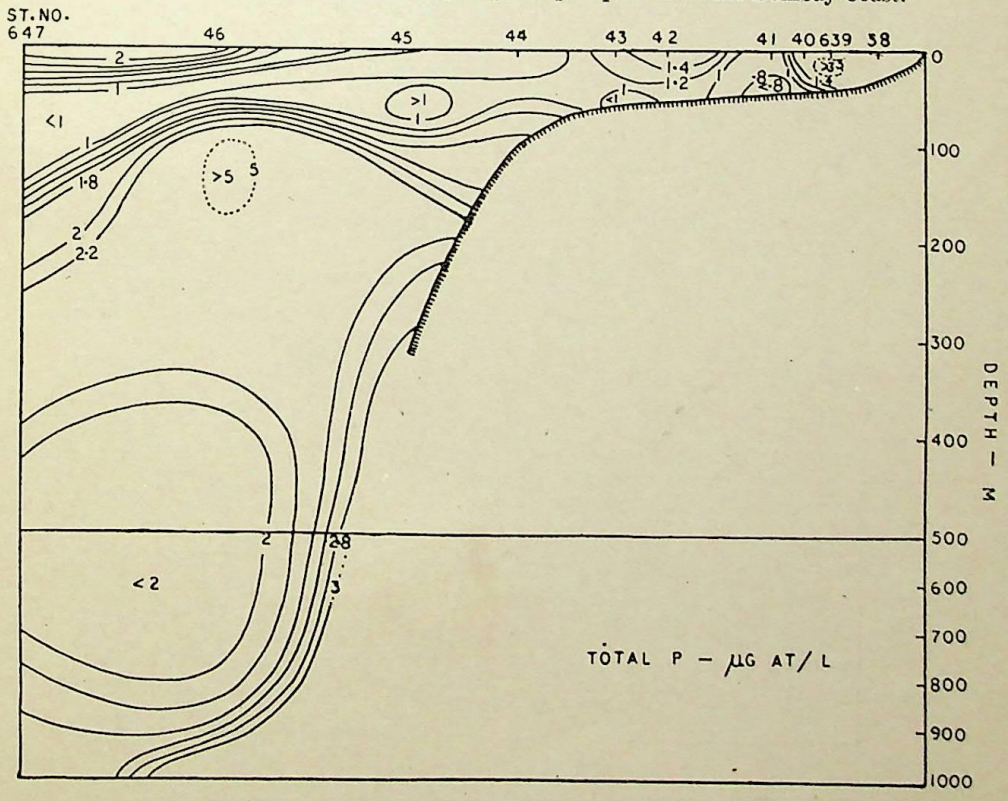

FIG. 3. Showing the distribution of total phosphorus off the Bombay Coast.

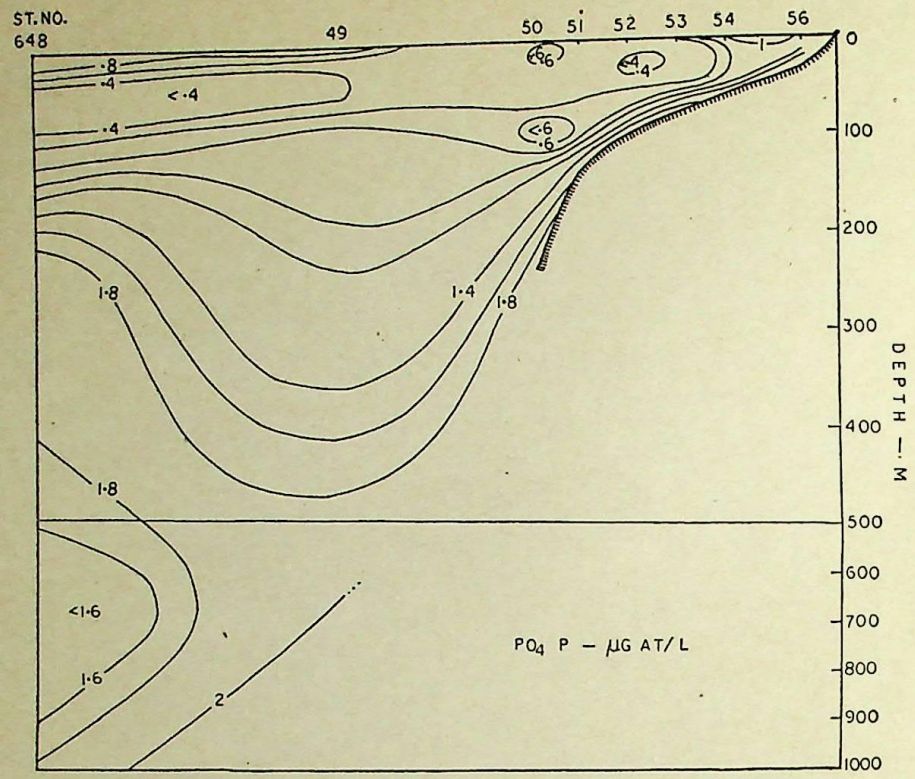

FIG. 4. Showing the distribution of phosphate-phosphorus off the Karwar Coast.

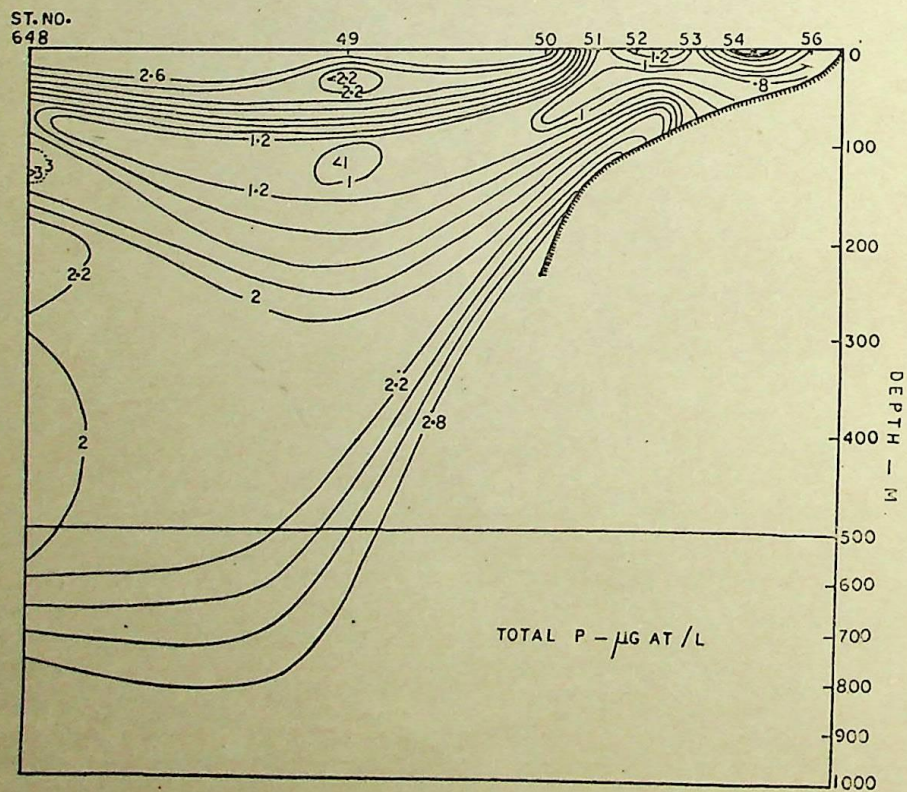

FIG. 5. Showing the distribution of total phosphorus off the Karwar Coast.

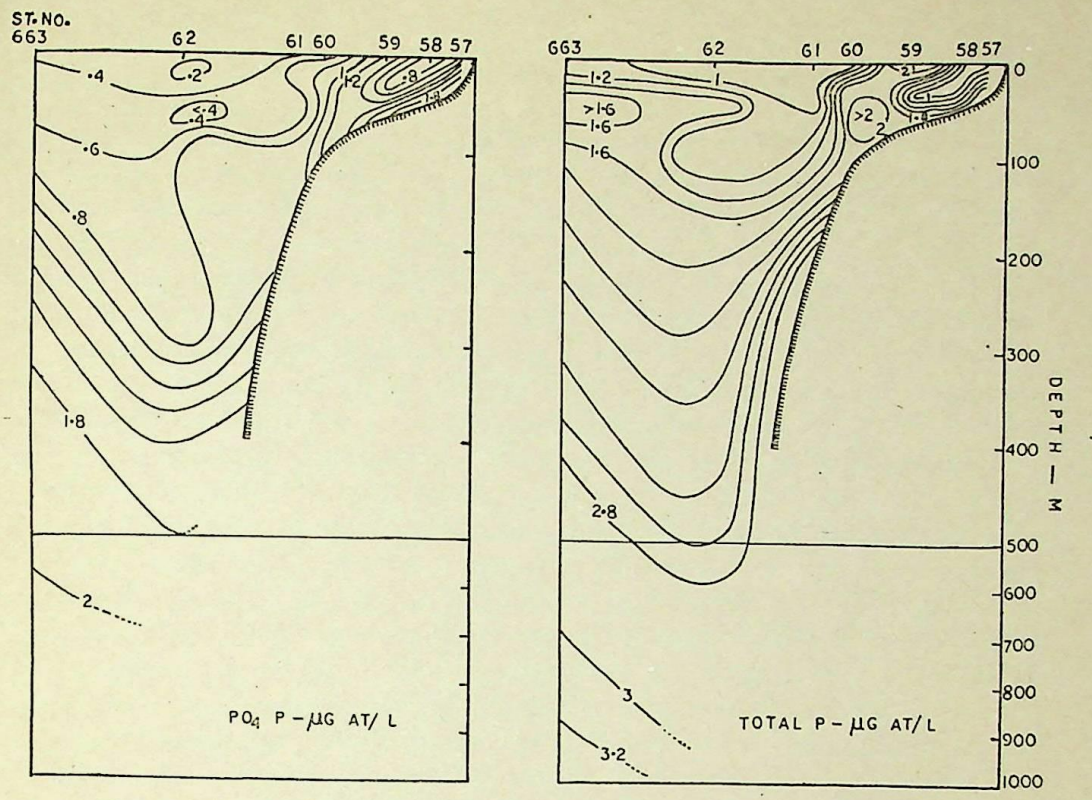

FIG. 6. Showing the distribution of phosphate-phosphorus and total phosphorus off the Mangalore Coast.

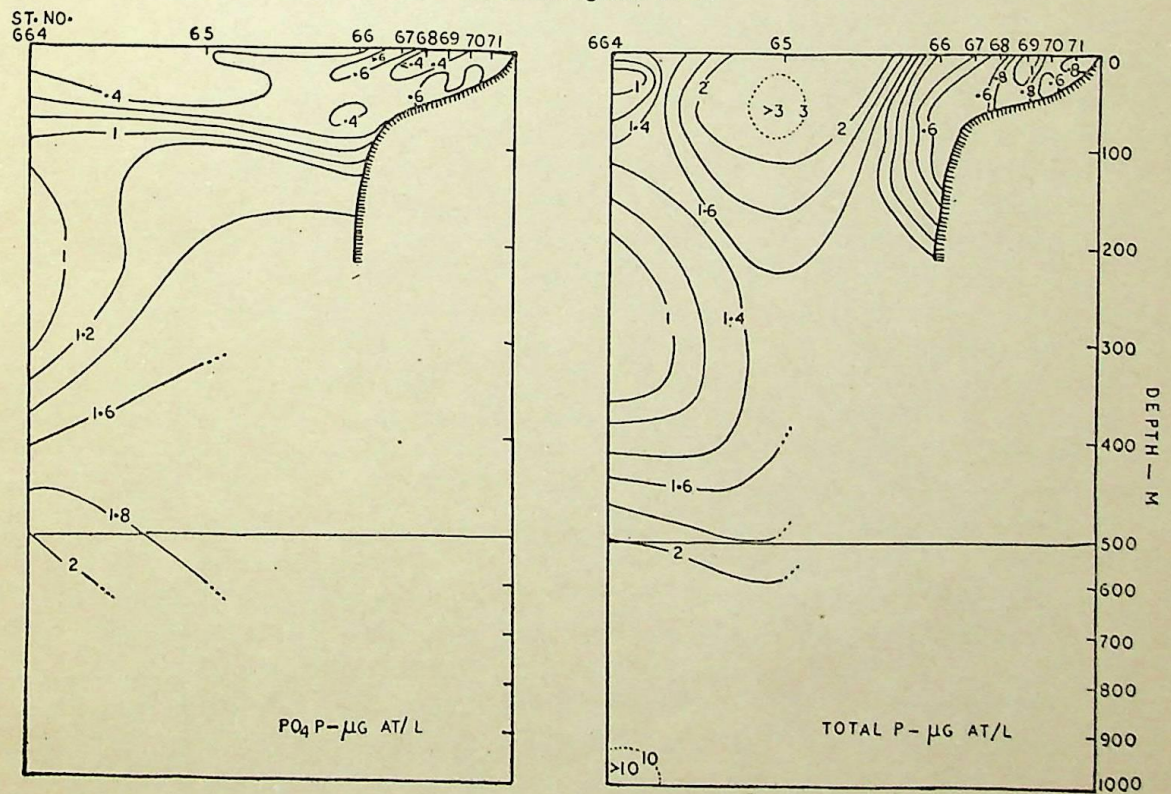

FIG. 7. Showing the distribution of phosphate-phosphorus and total phosphorus off the Kerala Coast.

will be presented along with other nutrients elsewhere. An examination of the profiles will indicate that there is a close similarity between inorganic and total phosphorus distributions in the three sections except along Cochin where similar distribution is restricted to the shelf region only. Total phosphorus values in the upper 1,000 m show wide variations. The range in the concentrations in the upper 500 m is 0·44–8·2 μg-at/l and at deeper levels it is 1·4–16·7 μg-at/l. It may, however, be mentioned here that concentrations exceeding 6 μg-at/l were recorded only off Cochin. Higher concentrations are normally encountered in the surface waters (upper 10 m) and in deep waters (below 500 m) with maximum in the latter. One characteristic feature in all the sections is the random cellular distribution of total phosphorus in the mixed zone (upper 100 m), containing either high or low concentrations. This feature can possibly arise due to entrapment of water bodies containing widely different concentrations of living and dead organic matter including soluble fractions during the process of mixing. This type of distribution is more pronounced in the shallow regions of the shelf.

Depthwise distribution of total phosphorus is irregular particularly in the upper 100 m and is more uniform below 500 m. Surface concentrations are generally increasing towards offshore. Clear maximum of total phosphorus is absent in the upper 1,000 m and from the available data of deeper levels (below 1,000 m) at few stations, it appears that its greater accumulation may be in between 1,000 m and 1,500 m.

Regional variation of the total phosphorus concentrations shows diverse trends. The concentrations in the upper 800 m are higher in the northern sections (off Bombay and Karwar) than those in the southern sections (off Mangalore and Cochin), but at deeper levels the trend seems to be reversed. Maximum surface concentration (4 μg-at/l) is found off Karwar in the offshore region. Off Cochin a greater concentration (> 10 μg-at/l) is found below 800 m as compared to the 2–4 μg-at/l found at the same depths in the other regions.

In the surface waters of the offshore region generally organic phosphorus forms the major fraction of the total. Apart from some random values of very high concentrations at few depths the organic phosphorus generally tends to decrease from 100 to 1,000 m. Even at 1,000 m the inorganic phosphorus was never equivalent to that of total phosphorus and the organic phosphorus amounted to 20–30 per cent of the total phosphorus. A comparison of the average organic phosphorus values in the shelf waters of different regions will reveal that maximum levels occur around Bombay (64 per cent of the total phosphorus). Along the Cochin area, it comes next (40 per cent) and off Karwar and Mangalore the concentrations are low, about 36–37 per cent of the total phosphorus. In waters beyond the shelf the percentage of organic phosphorus tends to increase gradually towards the south and the delineation becomes fairly marked between the northern and southern sections. The

average contents of organic phosphorus in the slope waters off Bombay and Karwar are 46 and 48 per cent of total phosphorus and off Mangalore and Cochin these are 54 and 56 per cent respectively.

The foregoing account indicates considerable variation in the regional distribution of the total phosphorus and its major fractions and even in a particular section the variations in the horizontal and vertical distributions are quite significant. Contrary to the fact that total phosphorus may serve as a useful index to identify water masses in general, the present data suggest that the total phosphorus loses its true conservative nature in the shallow and productive regions (shelf and slope) of the sea. Rochford (1958) during his investigations on the East Australian water masses in relation to total phosphorus observed that, in the region of turbulence extending to the bottom, anomalies in the distribution of total phosphorus occur. The high productive nature of the shelf and slope waters along the west coast of India perhaps renders the total phosphorus more a non-conservative property due to irregular distribution of all forms of organic matter aided perhaps by the random movement of the waters. Nevertheless the distribution of total phosphorus in the present instance broadly indicates only certain very distinct water movements and the fertility of the regions.

An examination of the total phosphorus data will reveal that the waters of the upper 800 m in the northern sections (off Bombay and Karwar) have a very high concentration of total phosphorus, much of which is in the organic form. In the southern sections (off Mangalore and Cochin) on the other hand the total phosphorus concentrations are relatively low and, excepting for the inshore region, the organic phosphorus remains predominant. Another notable feature is the total phosphorus concentrations in the slope region being consistently higher than those of the shelf and very high accumulation of total phosphorus (> 10 μg-at/l) is found off Cochin at 1,000 m and below. This high accumulation of total phosphorus (organic phosphorus 80 per cent) in the slope waters below 300 m is quite significant and it appears to have a good bearing on the fertility of the region in general (McGill 1964; Ryther and Menzel 1965). The high concentrations seem to be consistent with higher productivity of the region as a result of upwelling occurring during the southwest monsoon period. The particulate organic matter produced in the surface waters seems to be sinking to deeper layers without being mineralized completely. Total mineralization may be obliterated, perhaps, by the relatively lower oxidative nature of the water column. The high total phosphorus values in the upper 800 m in the northern section might also be related to the abundance of plankton and fish generally reported to be occurring during this period suggesting the probable sources for the standing levels of high phosphorus concentrations. Moreover the shelf, which is wide and shallow in the north, permits mixing to a considerable extent, distributing

phosphorus components from the sediments over a wide area. Examination of the vertical profiles indicates distinct convergence of the surface waters from the mid-shelf region extending over to a large area towards the slope region all along the coast. This feature is also in accordance with the distribution of other hydrographical factors. A general feature noticeable in the convergence regions is the presence of cells of high total phosphorus, comprising 60–80 per cent of organic phosphorus indicating the concentration of phosphorus of planktonic or detrital origin. From the standing levels of low inorganic and high total phosphorus in the surface layers, it could be inferred that the rate of regeneration is perhaps slow at least during the period under report and much of the regeneration activity appears to be limited to deeper layers as evidenced by greater accumulation of inorganic phosphates at these levels. However, mineralization is not complete even to 1,000 m at majority of the stations indicating the presence of significant quantities of organic phosphorus, which seems to be relatively more resistant to oxidation. This feature corresponds to some extent with those of recent investigations in the Pacific (Strickland and Austin 1960) and Atlantic (McGill 1964). Former authors suggest the presence of 'microstructure' of organic phosphorus distribution: a residuum which is highly resistant to process of mineralization. However, in the present instance, the presence of relatively high proportion of organic phosphorus at some locations suggests the possibility that the entire portion may not be the microstructure, but perhaps reflects on the general physical, chemical and bacteriological conditions of the upper 1,000 m controlling the rate of regeneration. The standing oxygen levels at these deeper layers are also too low to meet the oxygen demand for complete mineralization of the organic phosphorus and it is considered that some factors including the observed sinking phenomenon of the waters might be largely responsible in aiding faster sinking rate of particulate matter through the active regeneration zone (approximately between 200 m and 500 m characterized by low percentage of organic matter, Rochford 1962). More detailed investigations on the vertical distribution of different forms of phosphorus as a function of time may throw more light on this aspect.

Acknowledgements

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NOTE ON THE CUMULATIVE CELL, NUCLEUS, NUCLEOLI COUNTS IN GROWTH PATTERNS OF *PHYLLOXERA* GALL AND NORMAL GRAPE STEM SINGLE CELL CLONES IN TISSUE CULTURE

(PART I)

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In the present paper the following conclusions have been arrived at: (i) The growth of six single cell clones in terms of cell/nucleus/nucleoli multiplication appears to be more when RNA is incorporated in the medium than when DNA is incorporated in the medium. (ii) The logarithms to the base ten of the cumulative cell counts, nucleus counts and nucleoli counts can be fairly represented by $\log_{10} N_d = A+B(d-\bar{d})+C(d-\bar{d})^2$. (iii) The values of C are so irregular that no trend can be established.

INTRODUCTION

Studies from various standpoints of the *Phylloxera* gall and normal grape stem single cell clones in tissue culture have been made by Arya (1964), and Arya *et al.* (1962*a*, *b*, *c*). In the present paper we have studied the effects of incorporating nucleic acids (RNA and DNA) in the media on the cell size, nucleus size, nucleoli size, the cell counts, number of nucleus per cell, number of nucleoli per nucleus and their correlations, if any, in the growth of six single cell clones. This study will enable us to examine more closely the partly qualitative findings of Arya (1963) and his claim that the growth is correlated with the nucleic acid content.

MATERIAL AND METHODS

Six single cell clones, three from gall tissue incited by *Phylloxera vastatrix* Planch (GP_4—21, 8, 32) and three from normal grape stem tissue (GS_6—24, 81, 18) arranged in accordance to fast, medium fast and slow-growing characteristics, have been chosen for study. Four seed tissue transplants weighing approximately 35 mg were excised from three-week-old actively growing cultures. Each of these was grown in 6-oz prescription bottles on 40 ml of modified White's basal mineral salt agar medium supplemented with pancreatic casein digest (3·0 g/l), sucrose (2·5%), calcium pantothenate (2·5 mg/l),

NAA (1·0 mg/l), i-inositol (300·0 mg/l) and the content of RNA and DNA in the medium was as given in Table I.

TABLE I

Material Code	Content of RNA in the growing media	Material Code	Content of DNA in the growing media
I	0·0 mg/l	ID	0·0 mg/l
IV	40·0 ,,	IVD	40.0 ,,
V	400·0 ,,	VD	400·0 ,,
VI	4000·0 ,,	VID	4000·0 ,,

The media were adjusted to *p*H 5·9 to 6·0 after the addition of nucleic acids and then they were autoclaved. The cultures were grown in dark at 26°±2 °C and 55 per cent relative humidity. The harvests were made after 10, 20, 30 and 40 days. The material was fixed in acetoalcohol (absolute alcohol and glacial acetic acid in the ratio 1 : 1) and the fixed material was brought by Arya from Wisconsin, U.S.A. Five mg of this material was washed in distilled water and immersed in nearly 5 ml of an acid solution (5 per cent of chromic acid and 3 per cent of hydrochloric acid in the ratio of 1 : 1). The suspension was left at room temperature for a period of 24 to. 48 hours depending on the hardness of the tissue. The tissue was shaken vigorously for 5 minutes and repeatedly passed through a 10 ml syringe till the suspension appeared homogeneous. The cell suspension in chromic-hydrochloric acid was filtered. The Whatman filter paper No. 1 was used throughout the experiment. The cell mass left on the filter paper was washed several times with distilled water to remove chromic-hydrochloric acid. The cells so obtained were stained with 1 per cent aqueous solution of crystal violet and acetocarmine. The stained cell suspension was made in distilled water and a drop of this was taken out by a 10 ml pipette. Microculture technique (Jones *et al.* 1960) was used for making stained cell preparations.

For studying each material three microculture chamber slides were prepared. Three different counts were obtained from each slide, using three equal calibrated areas. Thus each preserved material yielded nine observations for each harvest which was made after 10, 20, 30 and 40 days for each fast, medium fast and slow-growing *Phylloxera* gall and normal grape stem clones. Thus quantitatively each material yielded 108 observations. In case the cell boundaries were not well defined the slide was rejected and a new slide was prepared to get nine observations for all counts. But when a newly prepared slide did not show any improvement, the mean of all the observations was considered as the cell counts. This was done for four materials, viz. $GP_4-21\frac{1}{40}$, $GP_4-32\frac{VD}{40}$, $GS_6-24\frac{IV}{40}$ and $GS_6-81\frac{VI}{10}$.

Observations

The cumulative cell, nucleus and nucleoli counts were made after a growth period of less than or equal to 10, 20, 30 and 40 days. For some of them the logarithms to the base ten of these cumulative counts were plotted on a graph paper against the number of days for which the material was grown. The logarithms to the base ten of these cumulative counts are found to be fairly parabolic with the concave side towards the axis of x and therefore the following empirical curve

$$\log_{10} N_d = A + B(d-\bar{d}) + C(d-\bar{d})^2$$

was fitted, where N_d is the average number of cells counted for a growth period of less than or equal to 10, 20, 30 and 40. Twenty-five days was considered to be the unit of days and d the number of days expressed as a multiple of 25 days as unit. A least squares solution was carried out to obtain the values of the constants A, B and C. The mean of these values for I, IV, V and ID, IVD, VD, VID was taken. The errors are probable errors from the mean. The equations of the curves appear to be as shown in Tables II and III.

Table II

Unit 0·001

		I, IV, V
Cell	GP_4-21	$\log_{10}N_d = (2558\pm143)+(520\pm55)\Delta d-(408\pm19)(\Delta d)^2$
	GP_4-8	$\log_{10}N_d = (2703\pm88)+(467\pm45)\Delta d-(346\pm106)(\Delta d)^2$
	GP_4-32	$\log_{10}N_d = (2233\pm53)+(526\pm46)\Delta d-(231\pm93)(\Delta d)^2$
	GS_6-24	$\log_{10}N_d = (2095\pm94)+(465\pm23)\Delta d-(213\pm48)(\Delta d)^2$
	GS_6-81	$\log_{10}N_d = (2247\pm117)+(431\pm58)\Delta d-(316\pm51)(\Delta d)^2$
	GS_6-18	$\log_{10}N_d = (2516\pm79)+(493\pm84)\Delta d-(364\pm92)(\Delta d)^2$
Nucleus	GP_4-21	$\log_{10}N_d = (1435\pm38)+(515\pm40)\Delta d-(204\pm48)(\Delta d)^2$
	GP_4-8	$\log_{10}N_d = (1474\pm95)+(542\pm35)\Delta d-(214\pm52)(\Delta d)^2$
	GP_4-32	$\log_{10}N_d = (1442\pm32)+(603\pm63)\Delta d-(192\pm28)(\Delta d)^2$
	GS_6-24	$\log_{10}N_d = (1435\pm44)+(546\pm29)\Delta d-(388\pm30)(\Delta d)^2$
	GS_6-81	$\log_{10}N_d = (1454\pm34)+(537\pm52)\Delta d-(308\pm48)(\Delta d)^2$
	GS_6-18	$\log_{10}N_d = (1401\pm46)+(565\pm53)\Delta d-(298\pm50)(\Delta d)^2$
Nucleoli	GP_4-21	$\log_{10}N_d = (1510\pm49)+(540\pm37)\Delta d-(274\pm24)(\Delta d)^2$
	GP_4-8	$\log_{10}N_d = (1534\pm146)+(514\pm25)\Delta d-(224\pm35)(\Delta d)^2$
	GP_4-32	$\log_{10}N_d = (1440\pm47)+(570\pm52)\Delta d-(161\pm45)(\Delta d)^2$
	GS_6-24	$\log_{10}N_d = (1500\pm28)+(546\pm53)\Delta d-(297\pm20)(\Delta d)^2$
	GS_6-81	$\log_{10}N_d = (1561\pm48)+(528\pm48)\Delta d-(304\pm28)(\Delta d)^2$
	GS_6-18	$\log_{10}N_d = (1464\pm64)+(547\pm27)\Delta d-(330\pm32)(\Delta d)^2$

where $\Delta d = (d-\bar{d})$

TABLE III

ID, IVD, VD, VID

Cell	GP_4-21	$\log_{10}N_d = (2721\pm 77)+(426\pm30)\Delta d-(251\pm19)(\Delta d)^2$
	GP_4-8	$,, = (2504\pm 40)+(320\pm63)\Delta d-(131\pm68)(\Delta d)^2$
	GP_4-32	$,, = (2414\pm 53)+(425\pm61)\Delta d-(169\pm62)(\Delta d)^2$
	GS_6-24	$,, = (2405\pm187)+(464\pm32)\Delta d-(184\pm51)(\Delta d)^2$
	GS_6-81	$,, = (2466\pm 66)+(458\pm39)\Delta d-(299\pm49)(\Delta d)^2$
	GS_6-18	$,, = (2497\pm 54)+(477\pm60)\Delta d-(392\pm52)(\Delta d)^2$
Nucleus	GP_4-21	$\log_{10}N_d = (1562\pm 71)+(459\pm26)\Delta d-(435\pm29)(\Delta d)^2$
	GP_4-8	$,, = (1567\pm 33)+(465\pm39)\Delta d-(276\pm63)(\Delta d)^2$
	GP_4-32	$,, = (1541\pm 81)+(423\pm54)\Delta d-(463\pm27)(\Delta d)^2$
	GS_6-24	$,, = (1553\pm 78)+(489\pm17)\Delta d-(292\pm37)(\Delta d)^2$
	GS_6-81	$,, = (1551\pm 46)+(479\pm37)\Delta d-(244\pm41)(\Delta d)^2$
	GS_6-18	$,, = (1555\pm 55)+(454\pm43)\Delta d-(495\pm16)(\Delta d)^2$
Nucleoli	GP_4-21	$\log_{10}N_d = (1576\pm 41)+(469\pm31)\Delta d-(320\pm74)(\Delta d)^2$
	GP_4-8	$,, = (1598\pm 45)+(429\pm31)\Delta d-(224\pm28)(\Delta d)^2$
	GP_4-32	$,, = (1541\pm 81)+(462\pm32)\Delta d-(289\pm75)(\Delta d)^2$
	GS_6-24	$,, = (1593\pm 71)+(479\pm20)\Delta d-(293\pm39)(\Delta d)^2$
	GS_6-81	$,, = (1599\pm 45)+(446\pm22)\Delta d-(262\pm22)(\Delta d)^2$
	GS_6-18	$,, = (1570\pm 44)+(459\pm35)\Delta d-(348\pm22)(\Delta d)^2$

The ratios of mean cell size to mean nucleus size and mean nucleus size to mean nucleoli size were obtained. These ratios were deduced (to each) for medium-fast growing material for GP_4 and GS_6 by taking the mean for fast, medium-fast and slow-growing material. The ratio between GS_6/GP_4 was determined from the data thus obtained. These values have been tabulated in Table IV below. The errors are probable errors from the mean.

It is very interesting to note that the empirical relation for these ratios can be tentatively written down as (GS_6/GP_4 Nu/Ni of I and IV) = (GS_6/GP_4 Cell/Nu of ID and IVD).

DISCUSSION

Cell, nucleus, nucleoli multiplication.—The values of A will of course depend on the number of cells/nucleus/nucleoli. The values of B when RNA was incorporated in the medium are systematically greater than the values of B when DNA was incorporated in the medium which shows that the growth in terms of cell/nucleus/nucleoli multiplication is more in RNA than in DNA. The values of C are so irregular that no trend can be established.

Cell.—The values of B for medium fast-growing gall GP_4-8 and normal grape stem GS_6-81 are the smallest amongst other values when RNA or

TABLE IV

	Material	GS_6/GP_4 Nu/Ni	GS_6/GP_4 Cell/Nu
Ratios	I/10	0·907±0·03654	0·687±0·15390
	I/20	1·018±0·03050	0·505±0·26383
	I/30	0·966±0·00695	0·765±0·10679
	I/40	0·966±0·00091	1·810±0·52439
	ID/10	1·023±0·02416	0·840±0·34277
	ID/20	0·959±0·06282	1·251±0·09453
	ID/30	1·075±0·00725	2·195±0·43488
	ID/40	1·195±0·07973	1·344±0·03835
	IV/10	1·199±0·10129	0·655±0·03322
	IV/20	0·855±0·02132	0·858±0·20445
	IV/30	1·871±0·05756	0·918±0·40921
	IV/40	0·949±0·02253	0·860±0·20808
	IVD/10	0·672±0·01903	0·757±0·05237
	IVD/20	0·925±0·08305	1·049±0·12400
	IVD/30	0·566±0·03171	1·014±0·10286
	IVD/40	0·651±0·13379	0·555±0·17437
Mean value	I	0·9675±0·01873	0·9418±0·26221
	ID	1·0630±0·04348	1·4075±0·22763
	IV	1·1935±0·05067	0·8227±0·21374
	IVD	0·7035±0·06690	0·8437±0·11340
Mean values of the materials treated with RNA and DNA	I and IV	1·0805±0·03470	0·8822±0·23797
	ID and IVD	0·8832±0·05519	1·1256±0·17051

DNA was incorporated in the medium. The obvious conclusion therefore is that the fast- and slow-growing GP_4-21, GP_4-32 and GS_6-24, GS_6-18 have more growth in terms of cell multiplication when either treated with RNA or DNA than their counterpart medium-fast growing GP_4-21 and GS_6-81.

Nucleus.—The values of *B* show gradual increase in GP_4 and GS_6 with the only exception of GS_6-81 when RNA is incorporated. But when DNA is incorporated in the medium the growth gradually increases as we pass from GP_4-21 to GP_4-8 and then decreases from GP_4-8 to GP_4-32. Whereas in GS_6 there is a gradual decrease as we go from GS_6-24 to GS_6-18.

Nucleoli.—The values of *B* are smallest for GP_4-8, GS_6-81. Therefore, we conclude that nucleoli multiplication is identical with cell multiplication.

However, the claim made by Arya (1963) that the growth is correlated with the nucleic acid content by the method of wet and dry weights cannot be fully verified in terms of the cell, nucleus and nucleoli multiplication.

This point in detail is being considered in terms of the cell, nucleus and nucleoli sizes and will be presented in the second part of the paper.

Acknowledgement

One of the authors (Mrs. S. P. G.) is grateful to Dr. H. C. Arya and Dr. B. Tiagi for encouragement.

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ELECTRON MICROSCOPIC STUDIES ON OSMOTICALLY RELEASED DNA FROM COLIPHAGE T-7

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Electron microscopy was done on T-7 bacteriophage DNA, as released by osmotic shock on water hypophase, following the 'protein monolayer' technique. Under certain conditions the osmotic shock method efficiently released the entire DNA content of the virus. The electron micrographs showed various conformations of the DNA genome. The number of molecules per phage was found to be unity. The molecular length, averaged from the measurements on 77 completely released molecules, was found to be $12{\cdot}72 \pm 0{\cdot}96\ \mu$ giving the molecular weight $24{\cdot}33 \pm 1{\cdot}84$ million.

Introduction

The technique of 'osmotic shock' has been very useful in inactivation studies of bacteriophages (Anderson 1949 and 1953; Leibo and Mazur 1966). The biology and chemistry of bacteriophages also have been extensively studied with the help of this method (Hershey and Chase 1952). In recent years, Kleinschmidt *et al.* (1962, 1965) introduced this method in electron microscopy for the studies of the DNA genomes from various viral sources. In this method the possible degradation of DNA molecules, due to chemical and mechanical handling involved in chemical extraction, can be avoided; further a very small amount of the sample is necessary, and the method is complete in itself.

Smallness of size of T-odd bacteriophages was supposed to be responsible for their resistance to osmotic shock (Anderson 1949). In the present work, attempts have been made to disrupt the T-7 bacteriophage by osmotic shock method in order to investigate the characteristics of the released DNA genome by electron microscopy. The micrographs furnished useful information about the conformation of the DNA, its length and the number of DNA molecules per bacteriophage.

Materials and Methods

Preparation of purified T-7 bacteriophage—Escherichia coli B, the host organism, was grown in nutrient broth at 37 °C in 1,000-ml batches on a rotary shaker. At the log phase of growth (titer 3×10^8/ml) the culture was infected with T-7 bacteriophage (the original sample of which is a gift from Prof. Arabinda Guha). The phage was purified and concentrated by differential centrifugation between the speeds of 20,000 r.p.m. for 2 hr and

30,000 r.p.m. for 1 hr. The bacterial debris was removed by centrifuging the phage at 8,000 r.p.m. for 15 min prior to differential centrifugation. Host RNA and DNA were removed by separate treatment with ribonuclease (50γ/ml) and deoxyribonuclease (5γ/ml). The phage was stored cold over chloroform in a solvent containing 0·1 M NaCl, 0·1 M PO_4-buffer, *p*H 7·4.

U.V. absorption, ultracentrifugal and electron microscopic characterization of T-7 bacteriophage—The U.V. spectrum of the phage, in the range of 200 to 300 mμ, was obtained with a PMQ II Zeiss Spectrophotometer. The phage was run in a Spinco model E analytical ultracentrifuge fitted with U.V. optics, using 30 mm aluminium centre-piece, 4° sector. The photographs were scanned with Hilger microdensitometer and S-value was calculated using 50 per cent boundary. A drop of well-dispersed T-7 phage solution was placed on specimen grids coated with carbon film. The phages settled on the film within a few seconds; then the solution was soaked away with a piece of filter paper. The grids were negatively stained with 1 per cent PTA (*p*H 7) and then seen under Siemens Elmiskop I at magnifications of 8,000×, 20,000× and 40,000×.

Electron microscopy of DNA released from osmotically ruptured T-7 bacteriophage—At first the technique of Kleinschmidt *et al.* (1962) was tried. But the modification introduced by Caro (1965) gave better results. The phage (concentration 5×10^9/ml) was mixed with 5–7 M ammonium nitrate solvent. 0·2 mg/ml of cytochrome-c was added to this and the suspension was kept at 4 °C for nearly an hour. The suspension was spread at room temperature on the surface of triple-distilled water, taken in a Langmuir trough. 0·2 ml to 0·5 ml of this spreading suspension was allowed to run down a wet glass slide which was inserted partially into the water surface in an inclined way. The movement of the invisible protein-film was visualized from the movement of tiny pieces of aluminium foil sprinkled previously about 5 cm away from the film. The use of the aluminium foil pieces instead of powder, which is generally used, was helpful in avoiding powder-contamination. The film was allowed to age for 10–15 minutes and then picked up by touching lightly carbon-coated electron microscope grids. The adhering water was soaked away from the grids by touching the surface of absolute alcohol for 20–30 secs. The grids were then shadowed with 5 Å of 10 per cent Ir-Pt alloy at an angle of about 6°. The metal was allowed to evaporate when the grids were rotating in the vacuum chamber. The electron micrographs were recorded in a Siemens Elmiskop I, operated at 60 kV and at a magnification of 8,000×.

Experimental Results

Fig. 1 is the absorption spectrum of the virus in the range of 200 to 300 mμ. The curve shows a minimum at 240 mμ and the ratio of O.D. (260)/O.D.

(280) equal to 1·53, characteristic of bacterial virus. These indicate also the purity of the phage preparation (Sinsheimer 1959). The curve shows two maxima, one at 259 mμ and the other at 206 mμ. These correspond to the X- and Y-peaks of a pure DNA sample (Basu and Das Gupta 1967). The sedimentation pattern of the purified phage preparation was characteristic of a homogeneous sample and its sedimentation coefficient extrapolated to zero concentration at 20 °C ($S^{\circ}_{20,w}$) was 427 S.

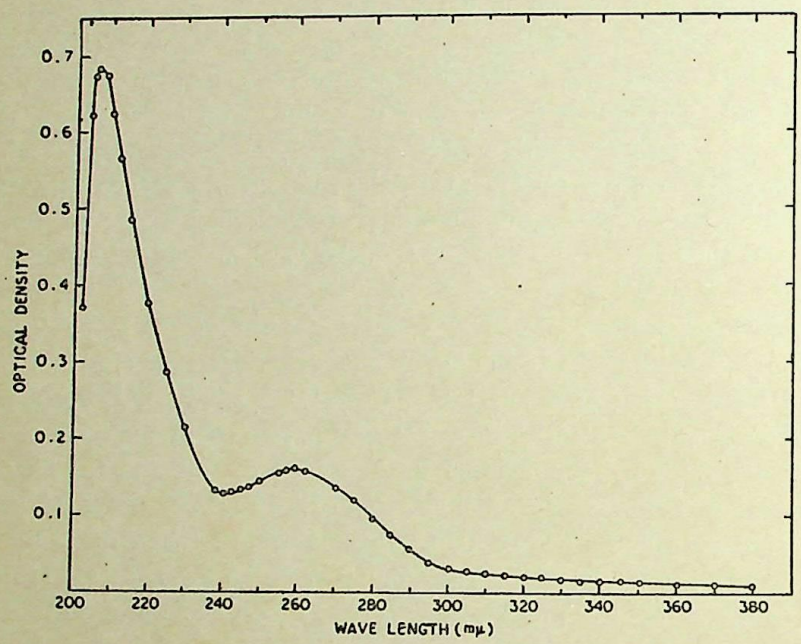
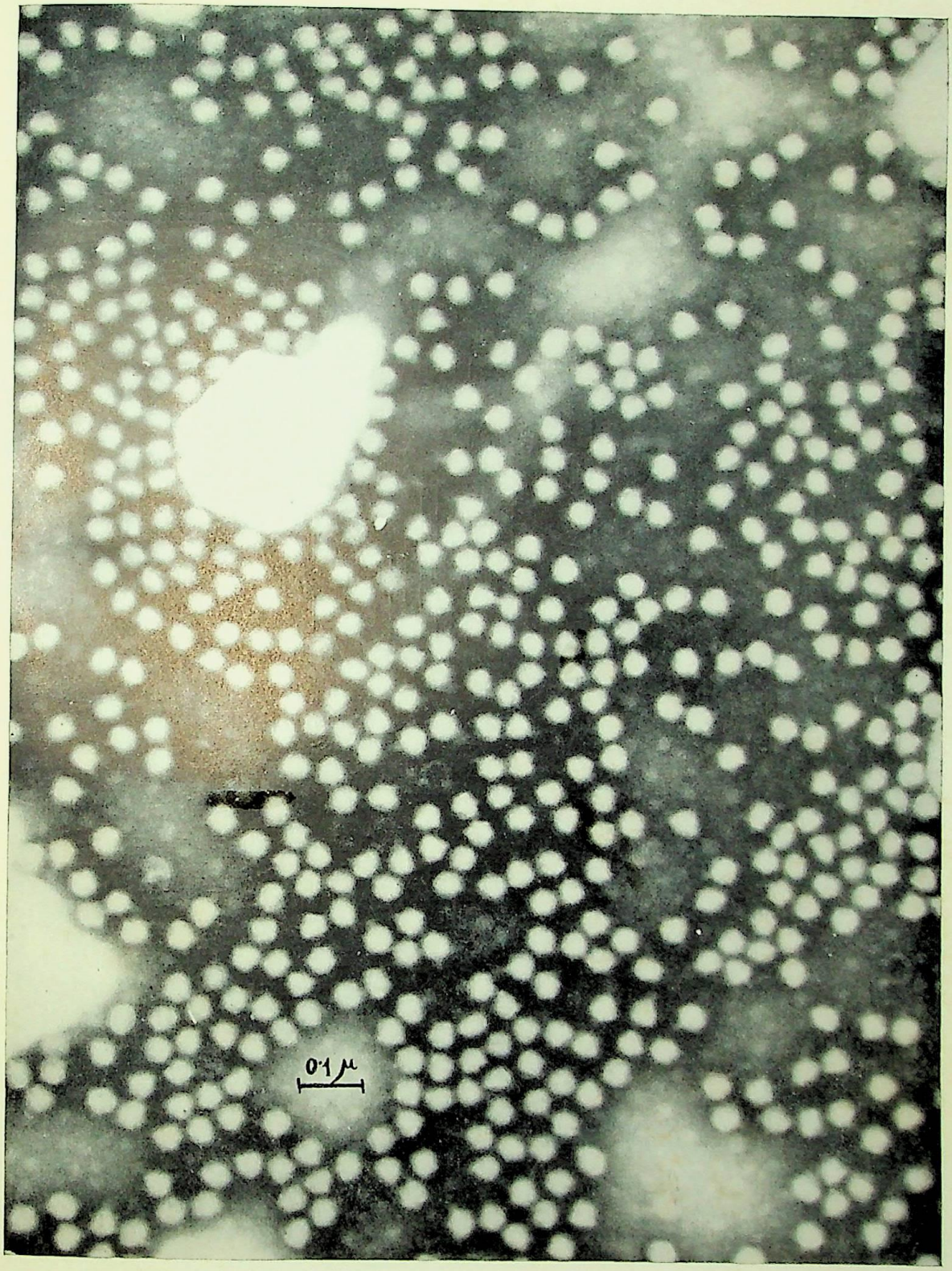


Fig. 1. Ultraviolet absorption spectrum of T-7 phage solution.

Plate I is an electron micrograph of the phage particles obtained by negative staining with phosphotungstic acid. Many of the phage particles have hexagonal cross-section and some of them show a diffuse tail. The particles have been measured across the flat faces giving the average diameter as 522±50 Å. Fig. 2 is a histogram showing the distribution of the measured diameters. Plate II is a characteristic micrograph of the osmotically released DNA. The field shows that all the viruses are disrupted, but the extent of release varies with different viruses.. The central portion of the field shows a completely released DNA molecule. When a drop of methyl alcohol was added to the virus-protein mixture just before spreading on the water surface, then the DNA release was more perfect, as most of the DNA molecules associated with the virus-ghosts are of full length. Plate III shows two completely released DNA molecules and two protein coats lying close to them.



Electron micrograph of T-7 phage obtained after negative staining with 1 per cent phosphotungstic acid (*p*H 7). Magnification 100,000×.

It is observed in Plate II that every DNA genome associated with the incompletely disrupted phages has one or two ends and never more. This indicates that each phage particle contains only one DNA molecule. Preparations containing very small concentrations of the phage always showed a single DNA molecule in the vicinity of a protein coat. Plate IV shows a full length DNA molecule passing through an apparently empty protein coat.

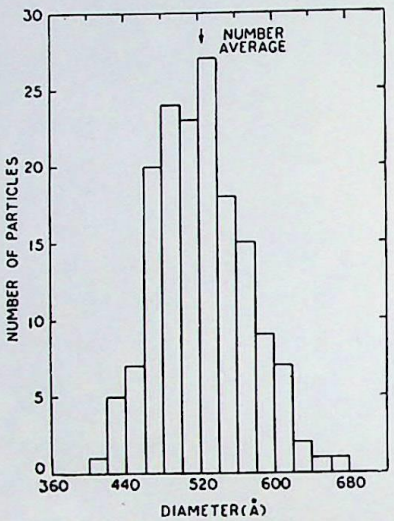
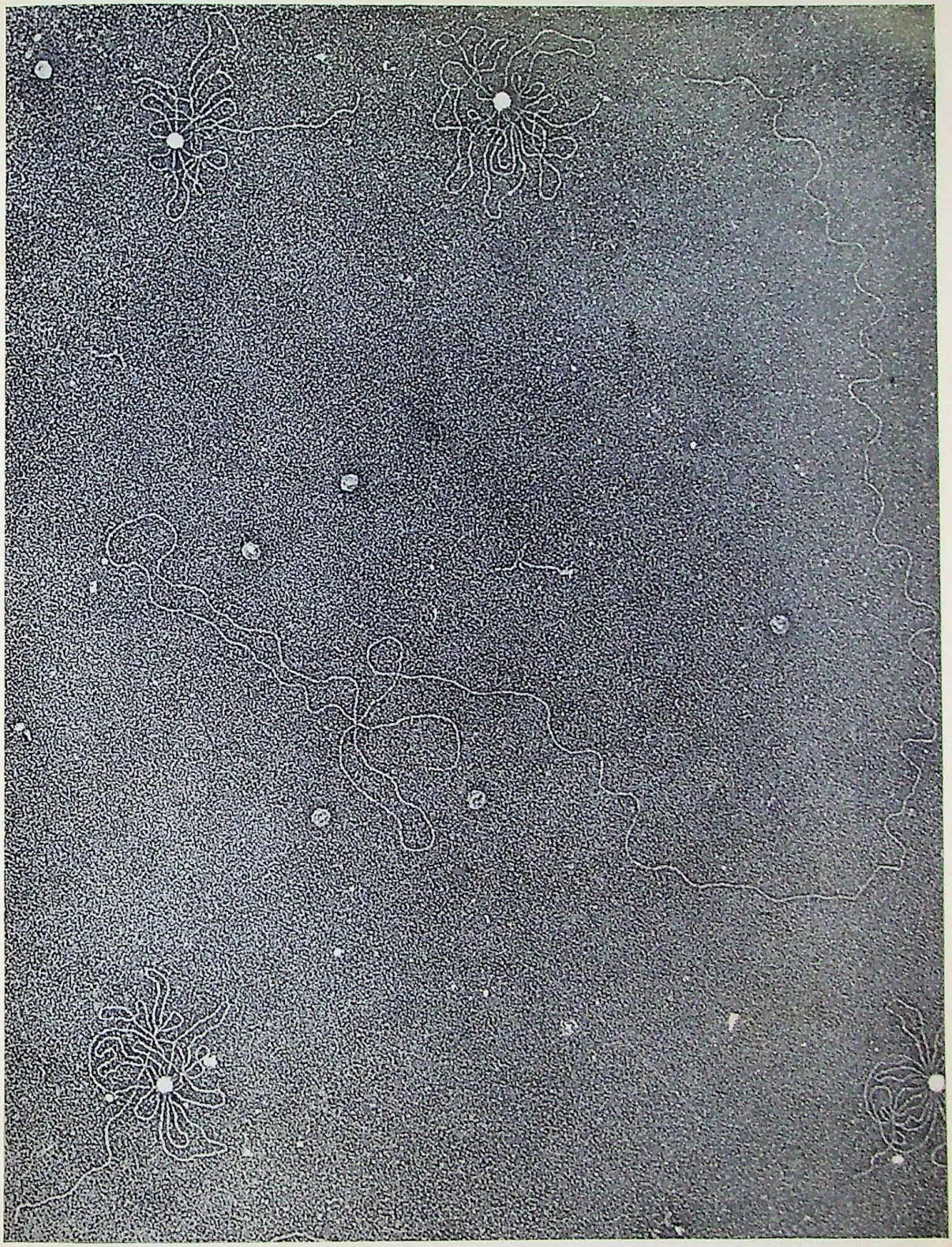


FIG. 2. Histogram showing the distribution of dimension of T-7 phage measured on electron micrographs obtained after negative staining with phosphotungstic acid.

The released DNA, as found associated with the incompletely disrupted phages, has in general the conformation as shown in Plate II. But in a few cases, a well-oriented conformation is found as shown in Plates V and VI.

A few molecules of DNA, released by osmotic shock method, were found to have clear denatured regions where strands have separated over short lengths. Plate VII shows such a molecule with a number of denatured regions marked by arrows.

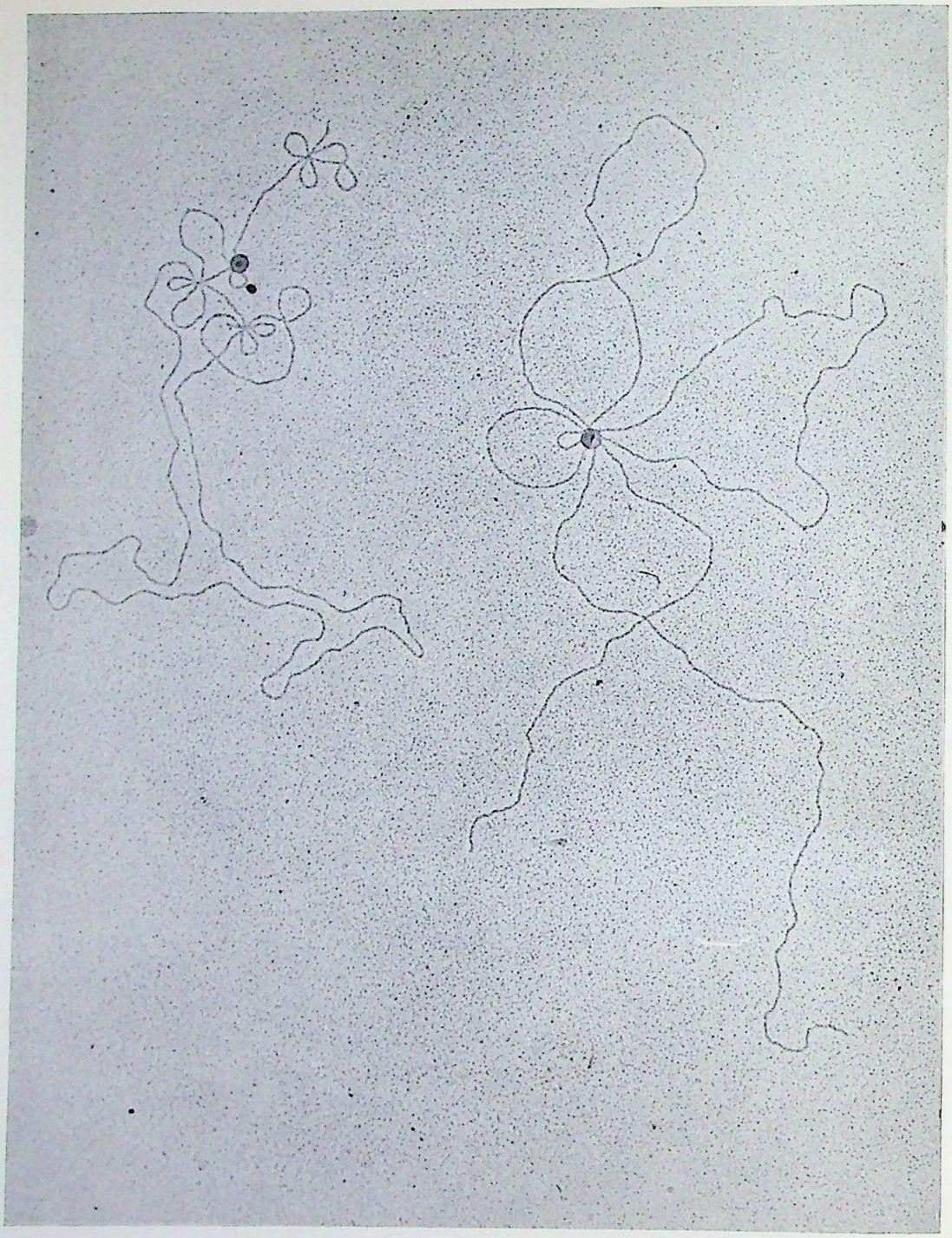
The completely released molecules have been measured by a map-distance measuring device. Fig. 3 is a histogram showing the distribution of the lengths of the molecules. The average length was found to be $12{\cdot}72 \pm 0{\cdot}96\ \mu$. The error in the measurements was calculated to be less than 10 per cent. Assuming that the DNA in wet condition has B-crystallographic configuration, the molecular length to molecular weight conversion factor becomes 1·913 million daltons per micron length (Langridge *et al.* 1960). The resulting molecular weight came out as $24{\cdot}33 \pm 1{\cdot}84$ million.



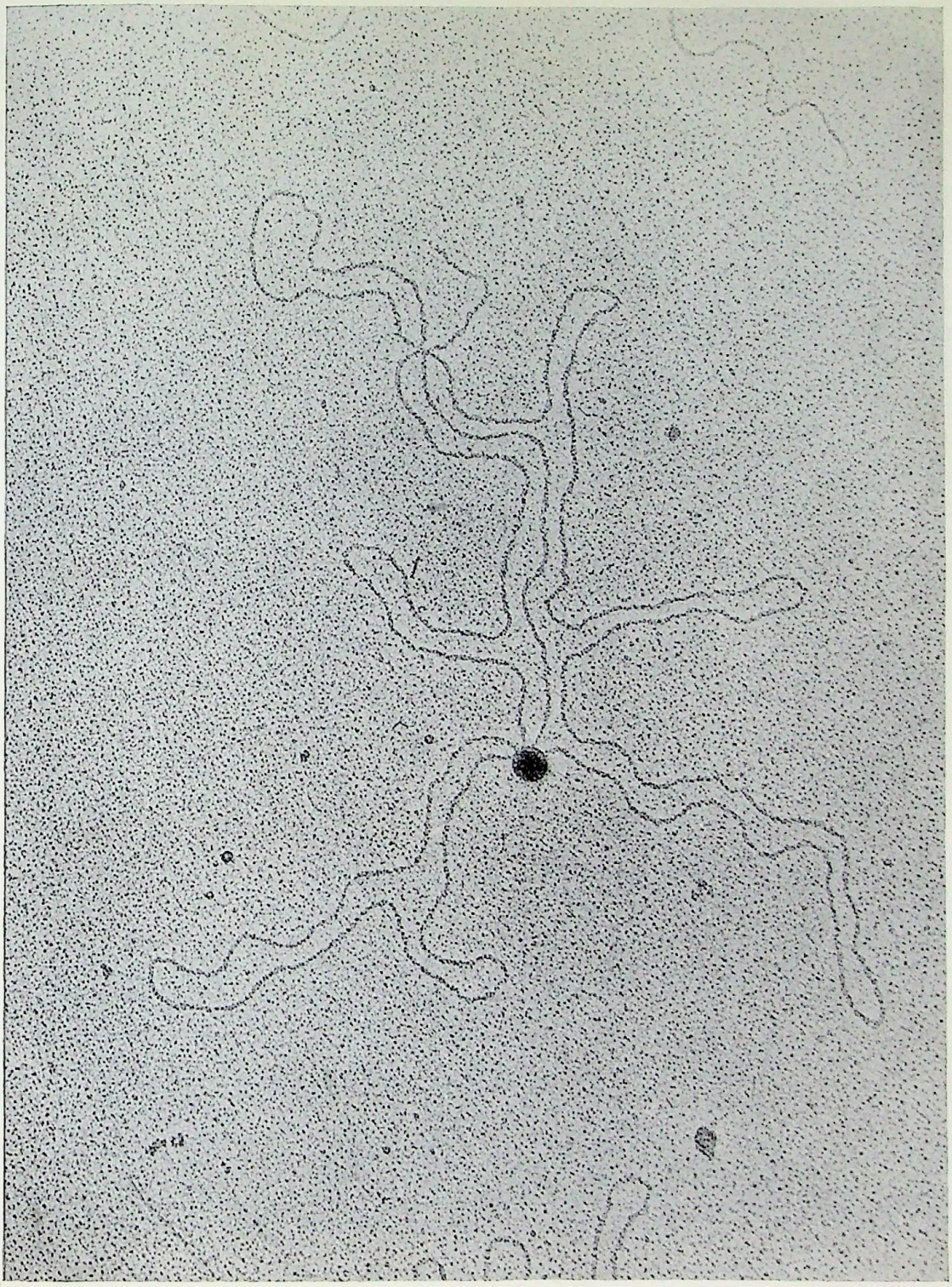
A typical electron micrograph of the osmotically released T-7 DNA. The field shows that all phages are disrupted, but to different extents. Magnification 44,000×.

This electron micrograph shows two completely released DNA molecules. The two protein coats are observed in the vicinity of the molecule. Magnification 64,000×.

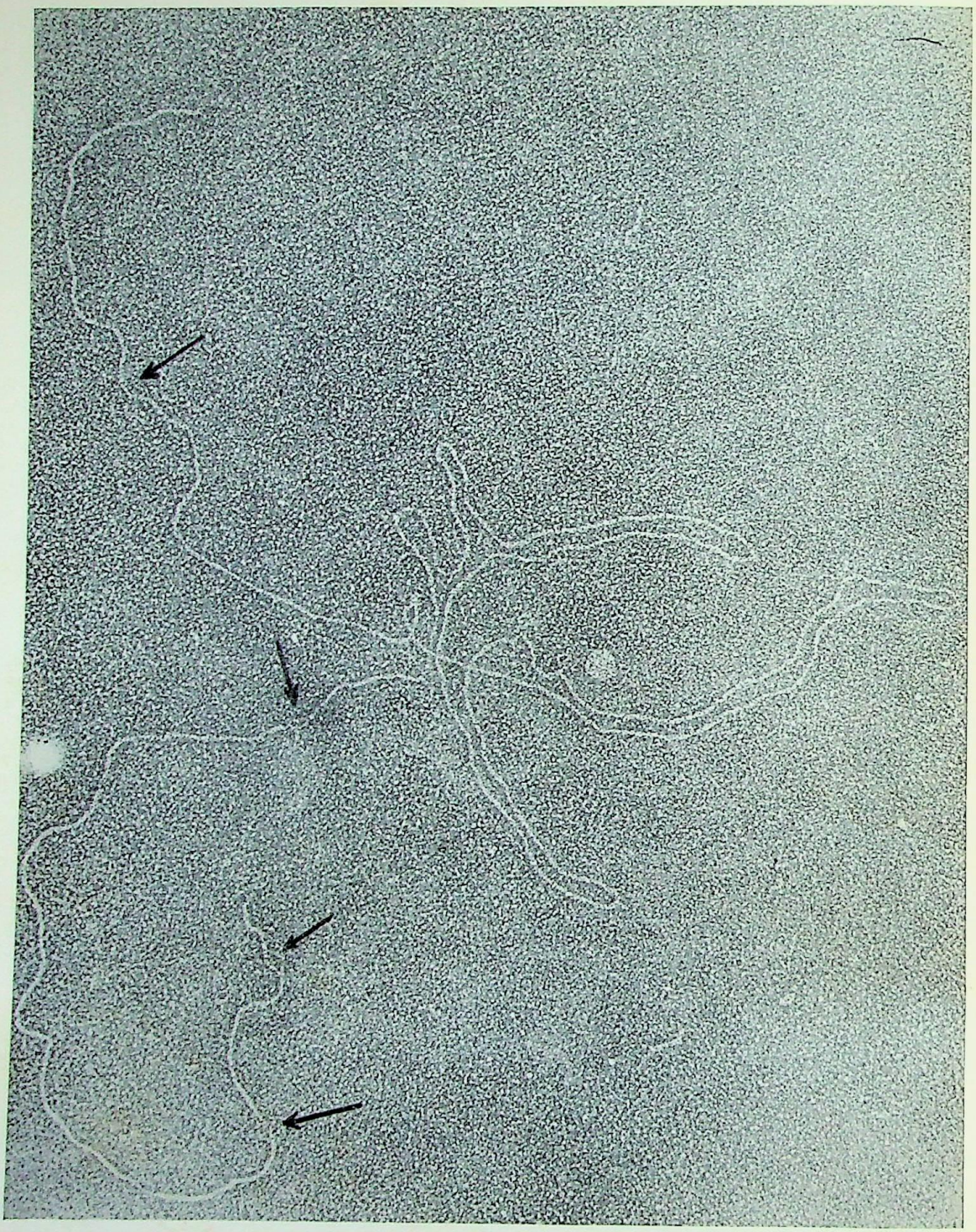
A full-length DNA molecule associated with its protein coat. Magnification 80,000×.



Two phages with their associated DNA genomes. One of the genomes shows a distinctive orientation. Magnification 48,000×.



A phage associated with incompletely released DNA genome which has a remarkable orientation. Magnification 80,000×.



A completely released DNA molecule with clear regions where strands have been separated (marked by arrows). Magnification 64,000×.

Discussion

T-odd bacteriophages are known to be resistant to osmotic shock (Anderson 1949). The present experiment showed that by proper choice of both the solute and the molarity of the solvent, T-7 bacteriophage could be disrupted osmotically to release its DNA content partially or fully. The phages, when incubated in 4–6 M-ammonium acetate, were found to release their DNA content only partially; but ammonium nitrate within the same molarity range resulted in effective release of the DNA. Caro (1965) made the same observations in the case of λ phage.

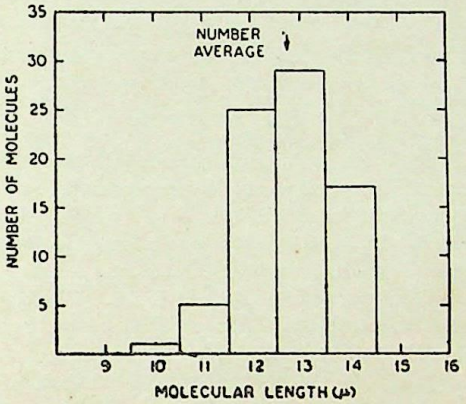


Fig. 3. Histogram showing the distribution of measured lengths of T-7 DNA molecules completely released by means of osmotic shock.

The length and the form of T-7 DNA, as obtained from osmotic shock, were the same as in the case of the DNA extracted by phenol (Misra *et al.* 1969). The same observations were also made by Kleinschmidt *et al.* (1962) in the case of T-2, Kleinschmidt *et al.* (1965) for SPV and by Caro (1965) in the case of λ phage. The superiority of the osmotic shock technique to the phenol extraction method, so far as the length and the form of the DNA molecules are concerned, lies in the fact that the former avoids various sorts of chemical and mechanical handling.

Tikchonenko *et al.* (1966) found spectrophotometrically that the DNAs of T-2 and S_D phages *in situ* were slightly hyperchromic compared to their extracted genomes. But this hyperchromicity vanished as soon as the DNAs of these phages were excreted into the medium. In our case, some electron micrographs showed denatured regions in the form of single-stranded loops along the duplex molecules but such denatured regions were absent from the electron micrographs of chemically isolated T-7 DNA molecules dispersed by Kleinschmidt technique under identical conditions (Misra *et al.* 1969).

However, slight local denaturation during the sample preparation procedure cannot be ruled out (Kleinschmidt *et al.* 1965). The lengths of 77 full-length, osmotically released T-7 DNA molecules were measured and the average length was found to be $12{\cdot}72 \pm 0{\cdot}96\ \mu$. In a previous communication (Misra *et al.* 1969) measurements on 46 such molecules were reported. The measurements of more molecules added to the accuracy and the reliability of the results.

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AMOEBIC LIVER ABSCESS PRODUCTION IN HAMSTERS BY INTRAPERITONEAL INOCULATION OF TROPHOZOITES OF *ENTAMOEBA HISTOLYTICA* WITHOUT LAPAROTOMY*

by G. P. Dutta, *Central Drug Research Institute, Lucknow*

(Communicated by B. N. Singh, F.N.I.)

(*Received 26 November 1968*)

A simple intraperitoneal method of producing experimental hepatic amoebiasis in hamsters without laparotomy is reported. After a small incision in the skin behind xiphisternum, hamsters were infected by injecting trophozoites of *Entamoeba histolytica* plus associated mixed bacteria through intact muscular layer of the abdomen. A satisfactory system of scoring hepatic lesions (0–4) has been described. The method is quick and requires no post-operative stitching. Out of sixty hamsters (weighing 40–120 gm), fifty-nine were infected with STA strain, the average hepatic lesion grade ranging from 3 to 4. Advantages of this method over other methods are discussed. Infection by six strains of *E. histolytica* produced acute liver lesions. This technique is being successfully used for screening potential systemic amoebicides. Emetine hydrochloride at 4 mg/kg completely cured 66% of the hamsters infected with STA strain and the average hepatic lesion grade was 0·7.

Introduction

Three main methods have been developed for the production of amoebic liver abscess in hamsters for chemotherapeutic studies—direct intrahepatic injection of *Entamoeba histolytica* trophozoites (Reinertson and Thompson 1951), intraperitoneal injection of amoebae in the region of umbilicus (Jarumilinta and Maegraith 1962) and liver region and gelatin sponge method (Jarumilinta 1966). In the last method a small piece of sponge is used to localize the amoebae and the sponge is placed between the middle and the left lobes of the liver at a point just above the gall-bladder. Jarumilinta (1966) found that the infectivity rate and average grade of lesions produced by these methods were approximately the same.

The methods developed, so far, involve laparotomy or damage to the liver, involving risk of secondary infection. The gelatin sponge method encourages the development of gelatin liquefying bacteria. Jarumilinta (1966) observed that bacteria associated with *E. histolytica* produced walled-off bacterial abscess containing yellowish liquefied material and partly liquefied gelatin between the left and middle lobes in all the 10 animals killed after 5 days. In the present communication, an intraperitoneal method of

* Communication No. 1280 from Central Drug Research Institute, Lucknow (India).

producing hepatic amoebiasis in hamsters without laparotomy is reported. It is being successfully employed for the screening of potential systemic anti-amoebic agents in this Institute.

Materials and Methods

Golden hamsters of either sex, weighing 40–130 gm, obtained from the Central Drug Research Institute colony, were employed in this work.

Six strains of *E. histolytica* (STA, C, B1, B1(502), RA and R) of the large race, obtained in culture from acute and carrier cases (Singh, Das and Saxena 1963; Das and Singh 1965), were maintained with mixed bacterial flora plus rice starch in modified Boeck and Drbohlav medium (M/40 phosphate buffer in 0·85 per cent (w/v) NaCl was used to dilute inactivated buffalo serum 1 : 8; *p*H 7·0). Growth of amoebae in fresh buffalo serum was better than in horse serum (Dutta and Mohan Rao 1966). In order to obtain healthy and actively motile amoebae, the method of Singh, Das and Saxena (1963) was followed. Twenty-four-hour-old culture of amoebae was pooled and washed a few times by centrifugation with diluted buffered buffalo serum in order to get rid of most of the bacteria. The amoebae were then inoculated into buffered serum in which mixed bacterial flora was allowed to grow for 16–18 hours and incubated at 37 °C for 18–20 hours. Hepatic infection with these amoebae gave better and more consistent results than the infection created by 24-hour-old culture of amoebae from modified Boeck and Drbohlav medium. The amoebae were kept at 37 °C throughout the operation.

For hepatic infection, about 0·5 cm long incision in the abdominal skin was made a little behind xiphisternum. Connective tissue, underlying the skin, was gently removed to expose the muscular layer of the abdomen. With the help of a pair of forceps, the muscular layer was raised from the viscera and 0·05–0·08 ml of the inoculum, containing 25,000–50,000 trophozoites, was dropped in the peritoneal cavity near the liver with a tuberculin syringe through the muscular layer. No post-operation stitching is required. The time taken to infect a hamster by this method is about half a minute, and the method is easy to manipulate. Moreover, this method ensures that the whole inoculum containing amoebae is dropped in the peritoneal cavity in the liver region and not accidentally injected into the viscera.

Infected hamsters generally died within 4–6 days. The liver lesions were cultured and also examined microscopically. With the help of a camera lucida, sketches of the liver showing lesions were made on a sheet of graph paper and the proportion of the liver infected was determined. The grading

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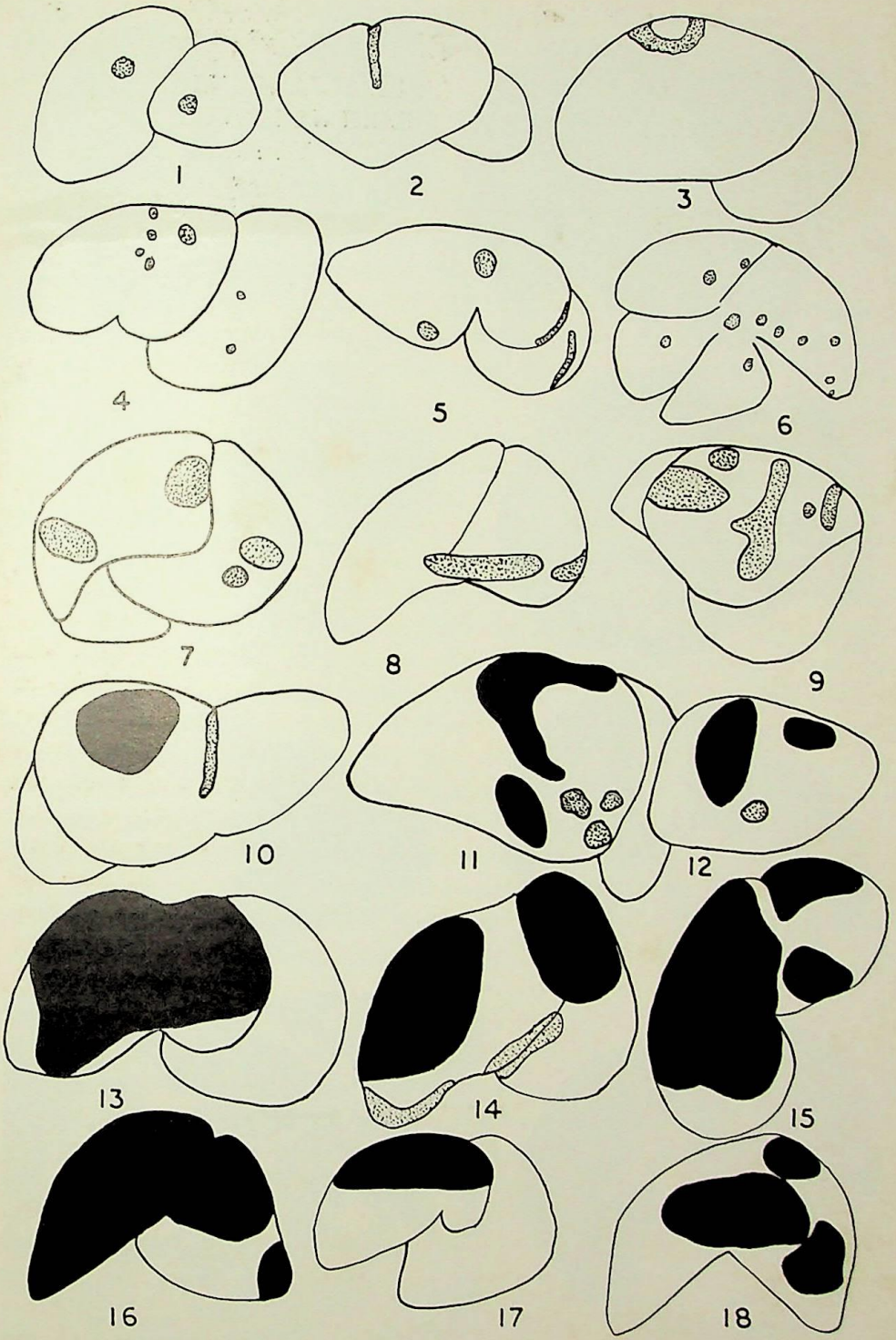
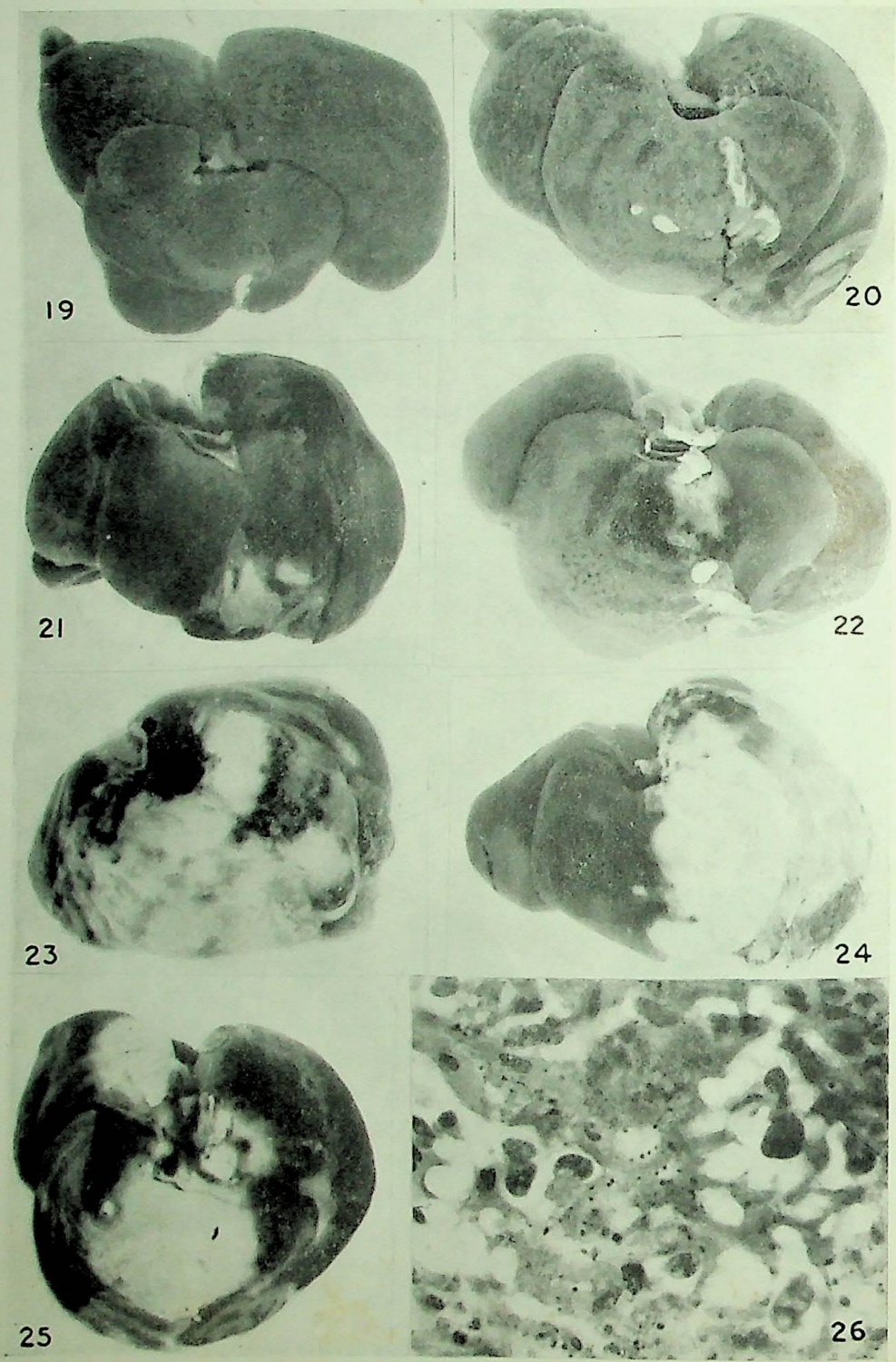
Figs. 1–18. 1–6, grade 1 amoebic liver lesions usually produced after 1–2 days of infection; 7–9, grade 2 lesions usually produced after 2–3 days of infection; 10–12, grade 3 lesions; 13–18, grade 4 lesions. Grade 3 and 4 lesions are usually produced after 3 or more days of infection.



E. histolytica (Strain STA) was used for infecting hamster livers.



E. histolytica (Strain STA) was used for infecting hamster livers.

of the liver lesions given below was based on more than 400 infected hamsters sacrificed after various intervals and also after the treatment of infected animals with emetine hydrochloride.

Grade O = No amoebic lesion, bacterial abscess may be present (Plate IX, fig. 19); 1 = Tiny superficial amoebic lesions covering up to 5 per cent of the liver surface (Plate VIII, figs. 1–6; Plate IX, fig. 20); 2 = 5–15 per cent of the liver surface showing lesions or inflammation with superficial necrosis (Plate VIII, figs. 7–9; Plate IX, fig. 21); 3 = Gross single or multiple lesions involving less than 25 per cent of the liver surface with extensive necrosis (Plate VIII, figs. 10–12; Plate IX, fig. 22); 4 = Acute single or multiple lesions covering more than 25 per cent of the liver surface with necrosis and pus, may be extending the whole thickness of one or more lobes (Plate VIII, figs. 13–18; Plate IX, figs. 23–25).

Active trophozoites were present in all the 1–4 grade lesions.

Ten hamsters were inoculated intraperitoneally by the above method with mixed bacterial flora of strain STA. 0·5 ml of the flora, without amoebae, was inoculated in 10 ml of buffered serum and incubated for 48 hours at 37 °C. 0·05 ml of this bacterial suspension was given to each hamster.

Amoebic infected livers were fixed in 4 per cent formaldehyde, sectioned and stained with Harris haematoxylin/eosin and Gram's stain. The sections were also stained with periodic acid-Schiff for the demonstration of bacteria as follows: Sections were put in 0·5 per cent aqueous periodic acid for 5 minutes (McManus 1956), washed with distilled water for 15 seconds, treated with Schiff's reagent (De Tomasi, 1936) for 15 minutes, washed in running water for 15 minutes, dehydrated and mounted in Canada balsam as usual. If desired, the sections were counterstained with Harris haematoxylin for 15 seconds and washed in running tap water before mounting.

In order to study the effect of emetine hydrochloride in hepatic amoebiasis, the drug was fed orally. First dose was given on the day of infection and one daily dose for four days after infection.

Results

The effect of the number of amoebae inoculated intraperitoneally in hamsters on the production of liver abscess is presented in Table I. In order to get nearly cent per cent infection of the liver, it is important to inoculate 25,000–50,000 amoebae per animal. It is clear from Table II that mixed bacterial flora present in the culture of *E. histolytica* (strain STA), inoculated intraperitoneally, did not produce bacterial lesions in any one of the hamsters.

Figs. 19–26. 19, bacterial liver abscess; 20, grade 1 amoebic liver lesion; 21, grade 2 lesion; 22, grade 3 lesion; 23–25, grade 4 lesions; 26, section of grade 4 amoebic liver lesion showing trophozoites, formaldehyde fixation and stained with Harris haematoxylin (×400).

Seven hamsters were immunized with formalized mixed bacterial flora of STA strain by two subcutaneous injections given after a week. After 14 days, the hamsters were inoculated with about 30,000 trophozoites per animal. Five animals produced 4 grade lesions and two 3 grade lesions, showing that immunization against bacterial associates had no effect on the production of liver lesions.

TABLE I

Effect of the number of amoebae (strain STA of E. histolytica*) inoculated intraperitoneally without laparotomy in hamsters on the production of liver abscess*

No. of amoebae	Weight of hamster	No. of hamster	Examination after inoculation (days)	Grade of hepatic lesion	Av.
1,000	40 gm	3	3(D), 4(D), 5(S)	3, 4, 0	2·3
2,000	50 gm	3	6(S), 6(S), 6(S)	4, 0, 0	1·3
5,000	50 gm	3	6(S), 6(S), 6(S)	3, 0, 0	1·0
10,000	50 gm	3	6(S), 6(S), 6(S)	4, 1, 0	1·7
25,000	50 gm	3	4(D), 4(D), 5(D)	4, 4, 4	3·7
50,000	50 gm	3	4(D), 5(D), 5(S)	4, 4, 4	4·0

D = died; S = sacrificed.

TABLE II

Effect of mixed bacterial flora of STA strain of E. histolytica *inoculated intraperitoneally without laparotomy in hamsters*

Weight of hamsters	No. of hamster	Examination after inoculation (days)	Presence of bacterial lesions in liver
60 gm	2	37(D), 38(D)	Nil
80 gm	4	17(D), 30(S), 30(S), 30(S)	Nil
80 gm	4	6(S), 6(S), 6(S), 6(S)	Nil

D = died; S = Sacrificed.

Production of liver abscess by strains of *E. histolytica* from acute and carrier cases is presented in Table III. All the six strains were able to produce 3–4 grade lesions. Out of sixty hamsters, weighing 40–120 gm, 59 were infected with the strain STA, the average hepatic lesion score ranging from 3 to 4.

When different weight hamsters were infected with strain STA intraperitoneally in the region of umbilicus after laparotomy by the method of Jarumilinta and Maegraith (1962), the hepatic lesions produced were very irregular and a number of hamsters were not infected (Table IV). When

TABLE III

Production of liver abscess in hamsters inoculated intraperitoneally with strains of E. histolytica *(50,000/animal) without laparotomy*

Strain	Wt. of hamster	No. of hamster	Examination after inoculation (days)	Grade of hepatic lesion	Av.
Acute Case					
STA	40 gm	4	6(D), 6(D), 6(S), 6(S)	4, 4, 4, 4	4·0
	50 gm	18	3(D), 4(D), 5(D), 5(D), 6(D), 9(D), 4(D), 4(D), 4(D), 4(D), 4(D), 11(D), 3(D), 4(D), 5(D), 5(D), 6(D), 9(D)	3, 4, 4, 4, 4, 4, 2, 3, 3, 4, 4, 4, 3 4, 4, 4, 4, 4	3·7
	60 gm	14	3(D), 5(D), 5(S), 5(S), 5(S), 6(D), 6(S), 6(S), 6(S), 4(D), 4(D), 4(D), 4(D), 11(D)	3, 4, 4, 3, 4, 4, 3, 4, 4, 3, 3, 4, 4, 4	3·7
	80 gm	14	4(D), 4(D), 4(D), 5(D), 5(D), 5(S), 5(S), 5(S), 5(S), 5(S), 6(D), 6(D), 6(S), 6(S)	3, 4, 4, 3, 4, 3, 4, 4, 4, 4, 4, 4, 4, 4	3·8
	100 gm	4	4(D), 6(D), 7(D), 7(S)	4, 4, 4, 4	4·0
	120 gm	6	4(D), 4(D), 5(D), 5(D), 6(S), 6(S)	2, 4, 4, 4, 4, 0	3·0
C	80 gm	4	4(D), 6(D), 6(D), 6(S)	4, 4, 4, 0	3·0
B_1	80 gm	6	3(D), 7(D), 8(S), 9(D), 9(D), 9(D)	4, 4, 4, 4, 4, 0	3·3
B_1 (502)	80 gm	6	3(D), 3(D), 6(D), 7(D), 8(S), 8(S)	3, 4, 4, 4, 0, 0	2·5
Carrier Case					
RA	80 gm	7	3(D), 4(D), 5(D), 5(D), 5(D), 5(S), 6(D)	3, 3, 4, 4, 4, 4, 4	3·7
R	80 gm	6	3(D), 3(D), 6(D), 6(D), 6(D), 8(S)	3, 3, 4, 4, 4, 0	3·0

D = died; S = sacrificed.

the inoculum, containing amoebae, was placed in the liver region according to the method of Jarumilinta (1965) after laparotomy, all the eleven hamsters were infected with the average hepatic score of 3·3–4·0 (Table V).

TABLE IV

Production of liver abscess by STA strain of E. histolytica *inoculated intraperitoneally (50,000 amoebae/animal) in the region of umbilicus after laparotomy by the method of Jarumilinta and Maegraith (1962)*

Wt. of hamster	No. of hamster	Examination after inoculation (days)	Grade of hepatic lesion	Av.
130 gm	4	4(D), 7(D), 8(D), 21(D)	4, 4, 0, 0	2·0
130 gm	3	9(D), 10(D), 30(S)	4, 2, 0	2·0
90–114 gm	6	6(S), 6(S), 6(S), 6(S), 6(S), 6(S)	0, 0, 0, 3, 0, 0	0·5
80 gm	4	8(D), 14(D), 30(S), 30(S)	0, 1, 0, 0	0·3

D = died; S = sacrificed.

TABLE V

Production of liver abscess by STA strain of E. histolytica *inoculated intraperitoneally (50,000/ animal) in the liver region after laparotomy by the method of Jarumilinta (1966)*

Wt. of hamster	No. of hamster	Examination after inoculation (days)	Grade of hepatic lesion	Av.
80 gm	4	4(D), 4(D), 4(D), 5(D)	4, 4, 4, 4	4·0
80 gm	4	5(D), 5(D), 8(D), 11(D)	4, 4, 4, 4	4·0
120 gm	3	4(D), 5(D), 5(D)	3, 3, 3	3·3

D = died.

It may be pointed out that in the technique requiring laparotomy for infecting hamsters in the umbilicus region, it was found that in a large proportion of the animals there was adhesion of viscera to the body wall and the formation of bacterial abscess containing pus. Inoculation in liver region after laparotomy resulted in the adhesion of the liver to the body wall at the point of incision. Bacterial abscess resulting in pus formation was also very frequently observed. In the intraperitoneal method of inoculation, employed in this work, without laparotomy, occasionally small bacterial abscesses containing pus develop along with the amoebic lesions. Bacterial abscesses are found only on the surface of the liver adjacent to the viscera. In animals in which no apparent amoebic infection was established or in infected animals treated with emetine hydrochloride, tiny bacterial abscesses are sometimes seen on the liver surface which may not show any adhesion to the viscera or the diaphragm (Plate IX, fig. 19).

TABLE VI

Chemotherapeutic effect of emetine HCl in hamsters infected with E. histolytica (*50,000 amoebae/animal*) *intraperitoneally without laparotomy*

Wt. of hamster	No. of hamster	Dose of emetine HCl (mg/kg/day)	Examination after inoculation (days)	Grade of hepatic lesion	Av.
40–60 gm	4	4	3(D), 4(D), 11(S), 11(S)	0, 3, 0, 0	
	4		6(S), 6(S), 6(S), 6(S)	2, 0, 0, 0	
	9		3(D), 4(D), 5(D), 6(D), 6(S), 6(S), 6(S), 6(S), 6(S)	1, 2, 2, 0, 0, 0, 0, 0, 0	0·7
	10		3(D), 3(D), 5(D), 6(S), 6(S), 6(S), 6(S), 7(S), 7(S), 7(S)	2, 2, 4, 1, 0, 0, 0, 0, 0, 0	

D = died; S = sacrificed.

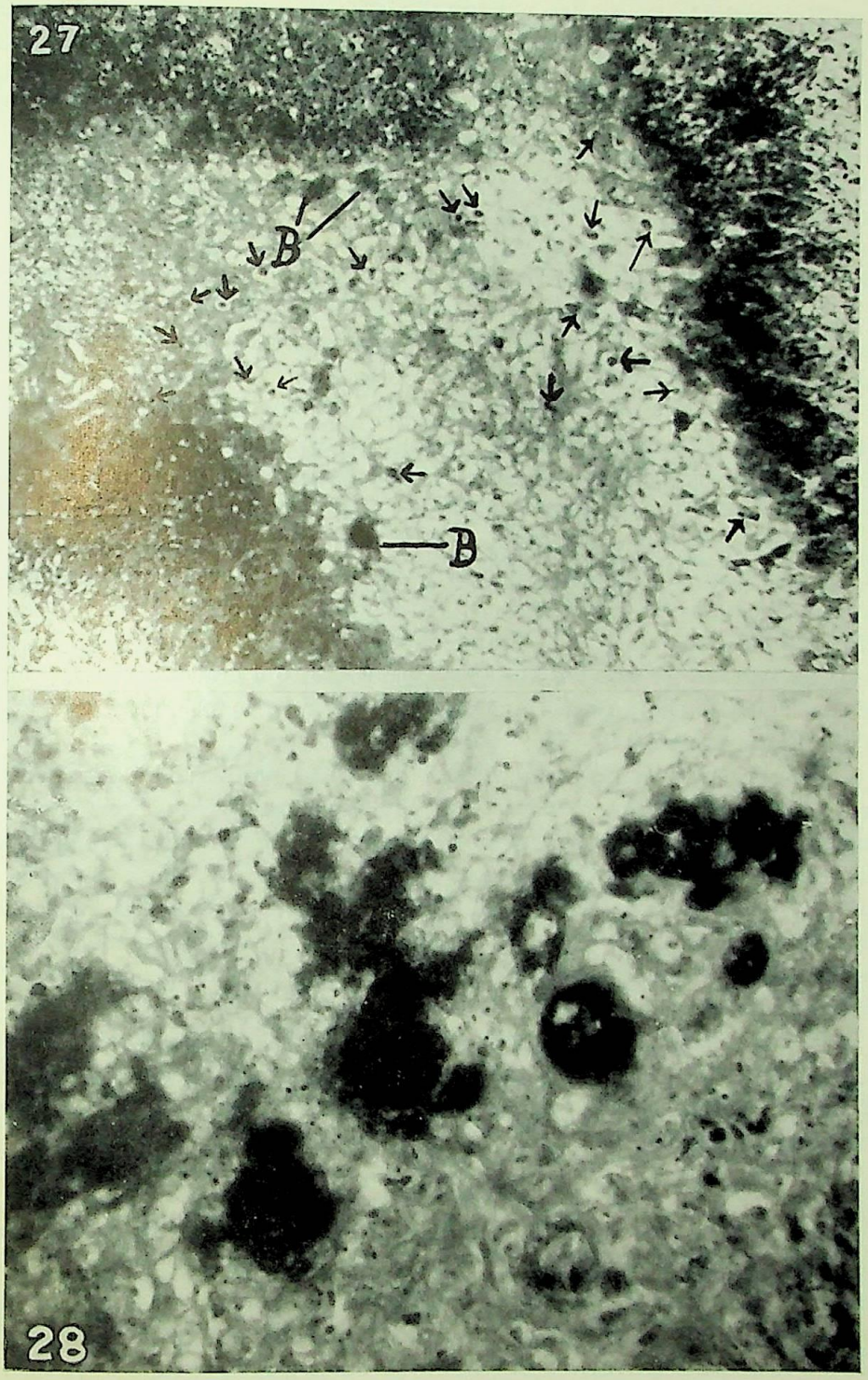
The chemotherapeutic effect of emetine hydrochloride in hamsters infected with strain STA is presented in Table VI. Sixty-six per cent of the hamsters were completely protected and the average hepatic lesion score was 0·7.

Histopathological study of the sections of 4 grade liver lesions, stained with Harris haematoxylin/eosin, showed an extensive necrosis of liver parenchyma. Where the area of necrosis merged with healthy tissue, a large number of trophozoites, accompanied with a marked leucocytic infiltration, predominantly of polymorphonuclear leucocytes, were noticed (Plate X, fig. 27). In the necrosed tissue, faintly staining debris of nuclei of the leucocytes was seen along with a few degenerating trophozoites, but no intact leucocytes (Plate IX, fig. 26). A few aggregations of Gram positive bacterial rods were also found in the necrosed tissue. They stained deeply in periodic acid-Schiff (Plate X, fig. 28) and faintly in haematoxylin preparations (Plate X, fig. 27). Smears made from the surface of the liver abscess revealed Gram positive and Gram negative bacteria. They stained with periodic acid-Schiff. In infected animals successfully treated with emetine hydrochloride, no necrosis of liver tissue or leucocytic and bacterial infiltration in the liver was found in the sections.

Discussion

In order to produce 100 per cent infection of *E. histolytica* in the liver of hamsters by intraperitoneal route, three factors are of paramount importance: (1) the trophozoites inoculated should be in healthy and actively motile condition, (2) they should be able to survive in order to colonize the host tissue and (3) the number of parasites inoculated. Unless these factors are properly standardized, the results cannot be consistent and reliable. Balamuth and Brent (1954) found that *in vitro* the survival and growth of *E. histolytica* were best in the complete absence of oxygen and were hampered at tension even as low as 0·1 per cent. The methods used by the previous workers (Reinertson and Thompson 1951; Jarumilinta and Maegraith 1962; Jarumilinta 1966) for creating infection in the liver after laparotomy do not seem to be ideal for the survival of the trophozoites. In this work, the trophozoites used for infecting the liver were in healthy and actively motile condition and were inoculated intraperitoneally in the liver region without laparotomy. The validity of this method can be judged by the results presented. All the six strains from acute and carrier cases produced high-grade liver lesions. There was cent per cent infection by STA strain in hamsters, weighing 40–120 gm, except in one animal weighing 120 gm.

Figs. 27–28. 27, section of grade 4 amoebic liver lesion showing heavy leucocytic infiltration and central area of necrosis, trophozoites are marked with arrows and bacterial aggregates as B, formaldehyde fixation and stained with Harris haematoxylin (×70); 28, above section stained with periodic acid-Schiff/Harris haematoxylin, to show the periodic acid-Schiff staining of bacterial aggregates which are seen in the area of necrosis (×400).



E. histolytica (Strain STA) was used for infecting hamster livers.

The method of infecting the liver by injecting trophozoites in the peritoneal cavity in the umbilicus region after laparotomy (Jarumilinta and Maegraith 1962) was not found to be satisfactory, especially when older hamsters were used. Liver region inoculation of the trophozoites after laparotomy, although produced high grade liver lesions, resulted in the adhesion of the liver to the body wall at the point of incision associated with the development of bacterial abscesses with pus. In gelatin sponge method, Jarumilinta (1966) found that the bacterial associate produced walled-off abscess on the liver surface in all the animals after five days. In the present method, bacterial abscesses were not produced on the liver surface by the bacterial associate. Bacterial abscesses occasionally developed, as reported in the text, when bacteria were injected along with the trophozoites.

It is apparent that the spread of amoebic infection by different methods—intrahepatic, intraperitoneal and gelatin sponge method—is bound to be different. A satisfactory method of scoring liver lesions is presented in this work. It differs in certain ways to the method of scoring described by Jarumilinta and Maegraith (1962). Their 1, 2 and sometimes 3 grade lesions (1–2 mm lesions more than ten) were found to be produced after one or two days of infection and have been put in the present system as 1 grade lesions. The grading adopted here is based on the percentage of the liver surface infected and also on the depth of the lesions. 3 to 4 grade lesions are more or less similar to that reported by them. It is simpler to record the proportion of the liver infected for grading the lesions.

Presence of bacteria in amoebic liver abscess produced by intrahepatic or intraperitoneal route of injecting the trophozoites has been reported (Reinertson and Thompson 1951; Williams 1959; Neal and Vincent 1956). In the present work, bacterial aggregates were also found in sections of infected liver in the area necrosed. It seems that the leucocytic infiltration surrounding the necrosed area is primarily due to amoebic invasion of the tissue. This histopathological appearance is not found after emetine hydrochloride therapy.

It is hoped that the method of producing hepatic amoebiasis reported in this paper will be of use to those engaged in the problem of chemotherapy of systemic amoebiasis and also in testing the virulence of *E. histolytica* from human acute and carrier cases.

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STUDIES ON PLANT-WATER RELATIONSHIPS *v.* INFLUENCE OF SOIL MOISTURE ON PLANT PERFORMANCE AND NITROGEN STATUS OF THE SHOOT TISSUE

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Three varieties of *Pennisetum typhoides* (viz. var. Hybrid, RSK and T55) were subjected to different moisture regime by watering to field capacity when the soil moisture tension increased to 1, 8 and 15 atm respectively. Plants maintained at field capacity served as control. Results narrate the changes in various characters of plants, changes in the concentration as well as in the absolute quantities of total, soluble and protein nitrogen contents of the shoot tissue at successive stages of growth, alterations in the nitrogen uptake rates and the grain nitrogen status. Vegetative growth was maximum in var. RSK but var. Hybrid showed the highest yield. Yield was depressed equally in all varieties under the 15 atm regime but var. Hybrid displayed superiority over others between the range of field capacity and 8 atm regime. Decrease in soil moisture brought about an increase in the nitrogen concentration of the shoot tissue at all stages of growth. There was a general decline in concentration with the age of the plant. A marked decrease in the absolute nitrogen content per plant, as well as in the nitrogen uptake rate, was noticed under 15 atm regime. These varieties showed two distinct peak periods of nitrogen uptake. The relative demands for nitrogen of these varieties at their different developmental stages have been outlined. Decrease in soil moisture increased the nitrogen content of the grains although the yield was adversely affected. Basic and applied implications of different findings have been discussed in the light of current concepts.

Introduction

There is a general unanimity amongst the contemporary reviewers (Wadleigh and Richards 1951, Stocker 1960, Vaadia *et al.* 1961) that the data so far obtained on the effects of soil moisture level on the nutrient accumulation of plants are rather equivocal. With special reference to nitrogen, certain workers (Gates 1957, Leamer *et al.* quoted by Wadleigh and Richards *loc. cit.*) have found that decrease in soil moisture leads to a decrease in the nitrogen content of the tissue, while others (Miller and Duley 1925, Emmert 1936, Janes 1950, Chen *et al.* 1964, Lahiri and Singh 1968) have found that the nitrogen content increases under conditions of water shortage. It may be speculated that decrease in water content may act to increase the concentration in the nutrient but this effect may be offset by such factors

as decreasing water turnover and hence restricted movement within the plant, or decrease in root growth. It may be possible again that these contradictory findings were due to varietal or plant differences.

However, there is no dispute about the fact that soil water shortage, in general, brings about adverse effects on the performance of plants, although the magnitude of effects, particularly in crops, may vary depending on the variety. It has been demonstrated (Tanaka *et al.* 1959) that varietal differences in growth and flowering may markedly change the nitrogen uptake pattern. Soil water shortage has also been found (Lahiri and Kharabanda 1965, Lahiri and Kumar 1966) to bring about significant changes in the expression of different vegetative characters. It will be logical to assume that such changes would also reflect on the nitrogen uptake behaviour. But our knowledge about the changes in nitrogen uptake due to variations in soil moisture is rather inadequate because of the foregoing controversies regarding the tissue nitrogen status. However, for the crop varieties, particularly for those which are grown in arid areas under rain-fed conditions, information regarding the changes in nitrogen uptake at successive stages of growth, as well as other changes which are brought about by soil moisture conditions, has an applied interest since they outline the relative demands for nitrogen at different growth stages of plants depending on moisture regime.

In nature, however, there is a continuous drift in the soil moisture reserve and thus the plants are subjected to tensions of different magnitudes. In view of these, attempts have been made here to study the relative performance of three varieties of pearl millet under conditions of soil moisture fluctuations (or cycles) from the field capacity to different specified tensions. The over-all impact of such moisture regime on the nitrogen status of the shoot tissue of these varieties has been studied at successive stages throughout their growing period. Moisture mediated changes in the rates of nitrogen uptake and the consequences on the nitrogen status of grains have also been outlined.

Materials and Methods

Three varieties of *Pennisetum typhoides*, viz. var. Hybrid 1, RSK and T55, were sown on 14 July 1967 in earthen pots (12 in dia.) containing sand, soil and farm-manure mixed in 1 : 2 : 1 proportions. Each pot contained about 7 kg of this mixture. Several seedlings were allowed to grow in the initial stages in each pot which were subsequently thinned to one plant per pot. Each pot received in the initial stages about 72 kg/ha of N as ammonium sulphate, 57 kg/ha of P_2O_5 as superphosphate and 19 kg/ha of K_2O as potassium sulphate.

The plants of all the three varieties were subjected to the following soil moisture regimes: (*a*) Plants maintained at field capacity (control);

(*b*) Plants watered to field capacity when the soil moisture tension increased to 1 atm ; (*c*) Plants watered to field capacity when the soil moisture tension increased to 8 atm; (*d*) Plants watered to field capacity when the soil moisture tension increased to 15 atm. Time of watering (in measured quantities) was adjudged for different treatments by periodic soil moisture determination from the root zone of 8 to 10 pots. Tension was determined from a curve relating the tension in atm with the per cent soil moisture for this soil. All the plants were watered to field capacity up to the 27th day from sowing and thereafter the differential watering commenced. Plants of var. Hybrid 1 were watered 35, 20 and 14 times under 1, 8 and 15 atm regime respectively. In var. RSK watering was done 34, 21 and 13 times under 1, 8 and 15 atm regime respectively. Similarly in var. T55, plants under 1, 8 and 15 atm regime were watered 32, 19 and 12 times respectively. Plants under field capacity were watered every day. Pots were kept in the open under natural conditions and a transparent plastic tent was put over the plants at the time of showers in order to prevent alterations in soil moisture.

Observations were recorded periodically on the growth in height, leaf number and tiller number throughout the growing period from the 26th day of sowing. Data were also collected on the number of days taken for ear emergence, length of ear, grain yield per plant and dry matter at harvest. All these observations were recorded on 15 plants (or pots) of each variety grown under each of the four moisture treatments. There were 50 other pots under each treatment from which samples were collected for nitrogen analyses and dry matter determinations.

For sampling purposes above ground portions of five plants under each treatment were collected on every 7th day between the 27th and the 90th day of sowing. After recording the dry matter, representative samples of the whole shoot portion was prepared by powdering in a ½ h.p. Willey grinding mill. The method of analysis for total, soluble and protein nitrogen was substantially the same as described in an earlier communication (Lahiri and Singh 1968). In all cases mean of three determinations has been presented. The nitrogen content per plant was derived from the dry matter weight and nitrogen concentration at different stages of growth was determined. The rate of uptake has been calculated as the amount of nitrogen (total) absorbed per day per plant for each seven days interval of growth between two sampling dates. The significance of the results has been adjudged by analysis of variance wherever necessary.

Results

Plant performance—The growth in height, leaf number and tiller number of the three varieties at successive stages has been presented in Fig. 1. It is of interest to note that the effects of soil moisture variation on the growth,

height and leaf number were discernible only at the later stages, but marked differences in tiller number could be found in all the varieties from early stages of growth. Again, a progressive decrease in growth, height and leaf number with decreasing moisture regime is often not consistent between field capacity to 8 atm, although plants under 15 atm tension showed large

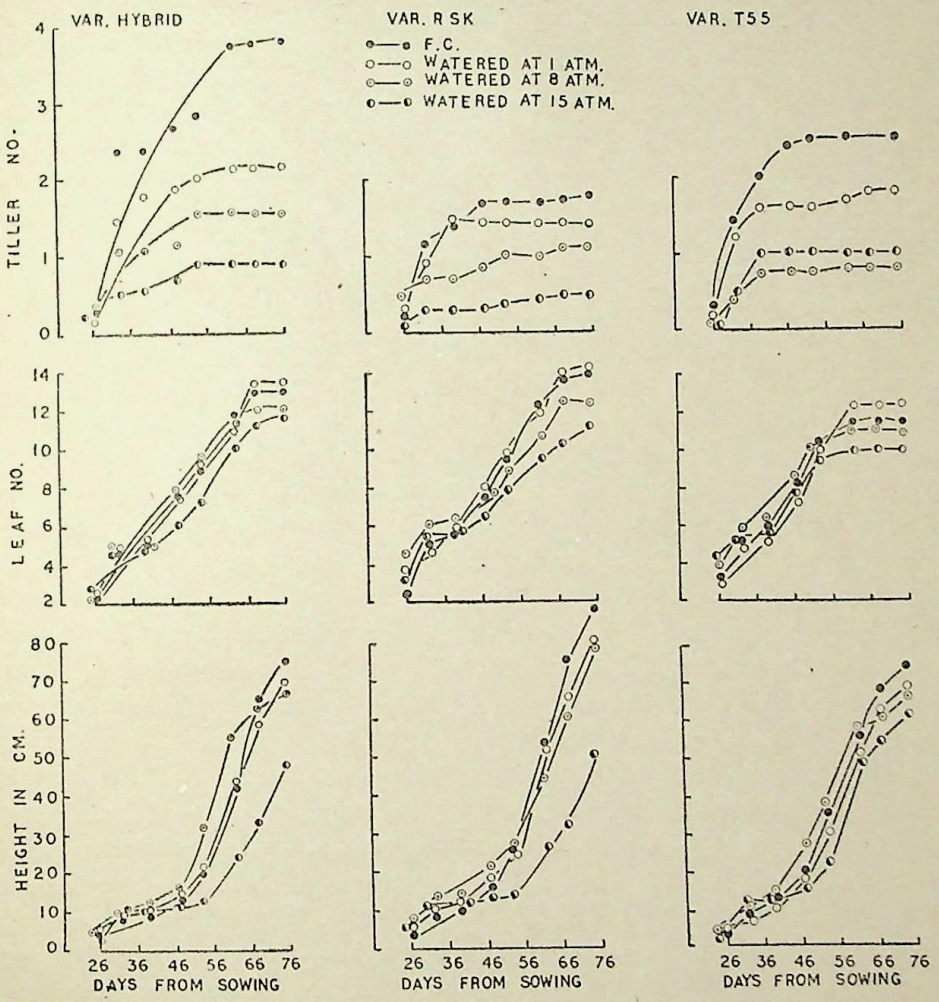

FIG. 1. Growth in height, leaf number and tiller number of var. Hybrid, RSK and T55 on different days from sowing.

and consistent inhibition. In var. T55 decrease in growth under 15 atm regime was relatively less as compared to the other varieties. Tiller number of var. Hybrid was much higher in comparison with the other varieties, although the decrease in tiller number with the progressive decrease in soil water status was found in all the varieties.

In order to adjudge the significance of the effects of moisture regime on plant performance and on the relative varietal traits, data on the final growth in height, leaf number (i.e. observations recorded on the 75th day of sowing), number of days taken for ear emergence, length of ear, grain yield per ear and dry matter at harvest were analysed by analysis of variance and the means have been presented in Table I. The marginal means for moisture treatments for final growth in height indicate that difference between field capacity and 1 atm regime was just significant at 5 per cent level while there was no significant difference between 1 and 8 atm. However, plants under 15 atm showed large and significant inhibition in comparison with other treatments. Marginal means for varieties indicate that plants of var. RSK showed significantly higher growth than the other two varieties but the difference between var. Hybrid and T55 was not significant. Significant interaction between variety and moisture regime suggested that soil moisture variation caused effects of different magnitudes in the three varieties. It may be found from the cell values that the variation in moisture regime between field capacity and 8 atm did not bring about any significant effect in any of the three varieties while at 15 atm, where the maximum adverse effect was produced, the magnitude of effect was significantly less in var. T55 as compared to others. The marginal means for moisture treatment for the character of leaf number suggest that there is a slight increase under 1 atm regime in comparison with the control. However, at 8 and 15 atm leaf number showed significant decrease. Marginal means for varieties indicate that var. RSK had the largest leaf number and the minimum was found in var. T55 while var. Hybrid held an intermediate position. Interaction between variety and moisture regime was not significant in this case. Marginal means for moisture regime under dry matter production at harvest indicate that the values decrease with decreasing moisture status, but the difference between field capacity and 1 atm regime and that between 1 and 8 atm were not significant. However, plants raised under the 15 atm regime had significantly low dry matter in comparison with the other treatments. Marginal means for varieties indicate that var. RSK produced significantly higher dry matter in comparison with the other two varieties. Difference between var. Hybrid and T55 was not significant. The number of days taken for ear emergence increased in general in all cases with increasing moisture tension. But the marginal means for moisture regime indicate that the differences between the range of field capacity and 8 atm regime were not significant. Only the value obtained at 15 atm regime was significantly less than all other treatments. Differences between varieties indicated that var. T55 required the minimum number of days for ear emergence while var. Hybrid and RSK had more or less similar values. Marginal means for moisture regime under the character of length of ear indicated that the values

TABLE I

Performance of var. Hybrid, RSK and T55 under different moisture regime

Treatments	Final growth in height in cm				Leaf number				Dry matter at harvest in gm/plot				No. of days taken for ear emergence				Length of ear in cm				Grain yield per ear in gm			
	Hybrid	RSK	T55	Mean	Hybrid	RSK	T55	Mean	Hybrid	RSK	T55	Mean	Hybrid	RSK	T55	Mean	Hybrid	RSK	T55	Mean	Hybrid	RSK	T55	Mean
1. Control kept at F.C.	75·6	88·3	74·4	79·4	13·1	13·6	11·9	12·9	7·8	8·7	6·9	7·8	69·5	68·2	61·2	66·3	13·6	17·2	15·5	15·4	3·33	1·55	1·31	2·06
2. Watered at 1 atm	70·0	80·3	68·0	72·8	13·6	13·9	12·1	13·2	6·8	7·7	5·8	6·8	70·9	68·8	60·9	66·9	12·7	16·5	14·3	14·5	2·86	1·36	1·25	1·82
3. Watered at 8 atm	67·1	78·6	66·8	70·8	12·2	12·5	11·4	12·0	5·7	7·5	5·5	6·2	71·3	69·5	60·0	66·9	12·3	15·3	13·9	13·8	1·91	0·85	0·87	1·21
4. Watered at 15 atm	48·3	50·5	61·7	53·5	11·9	11·1	9·9	11·0	3·4	4·2	3·2	3·6	77·1	80·5	65·7	74·4	11·5	13·4	12·2	12·4	0·45	0·42	0·36	0·41
Mean	65·3	74·4	67·7	—	12·7	12·8	11·3	—	5·9	7·0	5·4	—	72·2	71·8	62·0	—	12·5	15·6	14·0	—	2·13	1·04	0·95	—

	S. Em.	L.S.D.		S. Em.	L.S.D.		S. Em.	L.S.D.		S. Em.	L.S.D.		S. Em.	L.S.D.		S. Em.	L.S.D.	
		5%	1%		5%	1%		5%	1%		5%	1%		5%	1%		5%	1%
1. Marginal means for varieties ..	±2·0	5·5	7·2	±0·04	0·11	0·14	±0·3	0·82	1·08	±1·0	2·7	3·6	±0·31	0·85	1·12	±0·1	0·27	0·36
2. Marginal means for moisture regime	±2·3	6·3	8·3	±0·05	0·14	0·18	±0·37	1·02	1·33	±1·1	3·0	4·0	±0·3	0·82	1·80	±0·12	0·33	0·43
3. Cell values	±4·0	11·0	—	±0·4	—	—	±0·65	—	—	±2·0	—	—	±0·6	—	—	±0·2	0·55	2·72

TABLE II

The concentrations of total, soluble and protein nitrogen in the shoot tissue of var. Hybrid, RSK and T55 at their successive stages of growth under different soil moisture regime

Moisture regime	Variety	Number of days from sowing																														
		27			34			41			48			55			62			69			76			83			90				
		Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %	Total N %	Soluble N %	Protein N %
1. Plants maintained at field capacity	Hybrid	3·1909	0·7726	2·4183	1·8375	0·3843	1·4532	1·4437	0·3090	1·1347	1·3225	0·2979	1·0246	1·3000	0·3421	0·9579	0·9597	0·2847	0·6750	0·8996	0·2745	0·6248	0·8932	0·2711	0·6221	0·8521	0·2647	0·5874	0·8595	0·2697	0·5898	
2. Watered when soil moisture decreased to 1 atm.	—	—	—	—	1·9540	0·3974	1·5566	1·5050	0·3157	1·1893	1·2669	0·2847	0·9822	1·3671	0·3378	1·0293	1·0118	0·3140	0·6978	0·9269	0·2648	0·6621	0·9170	0·2712	0·6458	6·8983	0·2795	0·6188	0·8909	0·2852	0·6057	
3. Watered when soil moisture decreased to 8 atm.	—	—	—	—	2·2190	0·4547	1·7643	1·8705	0·3390	1·5314	1·5434	0·3157	1·2277	1·4533	0·3618	1·0915	1·4318	0·3937	1·0381	1·4000	0·3855	1·0145	1·4533	0·4050	1·0483	1·1697	0·3302	0·8395	1·0599	0·3353	0·7246	
4. Watered when soil moisture decreased to 15 atm.	—	—	—	—	2·2459	0·4407	1·8052	2·4551	0·4298	2·0253	2·3264	0·4254	1·9010	2·0720	0·4305	1·6415	1·6898	0·4011	1·2887	1·6655	0·3974	1·2681	1·6424	0·4451	1·1973	1·6185	0·4523	1·1662	1·6112	0·4772	1·1340	
1. Plants maintained at field capacity	RSK	3·0152	0·7859	2·2299	1·6800	0·3805	1·2995	1·3447	0·3313	1·0134	1·2812	0·3518	0·9294	1·2942	0·2972	0·9970	1·1356	0·3011	0·8345	1·1317	0·3117	0·8200	1·0808	0·3103	0·7705	0·9397	0·3125	0·6272	0·8547	0·2864	0·5683	
2. Watered when soil moisture decreased to 1 atm.	—	—	—	—	1·8015	0·4168	1·3847	1·3810	0·3406	1·0404	1·2588	0·3157	0·9431	1·2420	0·3023	0·9397	1·1707	0·3009	0·8698	1·3487	0·3105	1·0382	1·3114	0·3137	0·9977	0·9138	0·2858	0·6280	0·9043	0·2840	0·6203	
3. Watered when soil moisture decreased to 8 atm.	—	—	—	—	1·8355	0·4185	1·4170	1·8015	0·4454	1·3561	1·4988	0·3282	1·1706	1·4685	0·3498	1·1187	1·4000	0·3405	1·0595	1·5034	0·3776	1·1258	1·3866	0·3137	1·0729	1·3324	0·4411	0·8913	1·2880	0·3745	0·9135	
4. Watered when soil moisture decreased to 15 atm.	—	—	—	—	2·1861	0·5036	1·6825	2·0604	0·4695	1·5909	1·9764	0·4238	1·5226	1·9743	0·4250	1·5493	1·9675	0·4381	1·5294	1·9333	0·4398	1·4935	1·9066	0·5400	1·3666	1·8755	0·5428	1·3327	1·7158	0·5052	1·2106	
1. Plants maintained at field capacity	T55	3·3841	0·9357	2·4484	2·0564	0·4882	1·5682	1·6258	0·3467	1·2791	1·2684	0·3360	0·9324	1·2050	0·3427	0·8623	1·0681	0·3069	0·7612	1·0105	0·3054	0·7051	0·9594	0·3262	0·6332	0·8696	0·3351	0·5345	1·0105	0·4117	0·5988	
2. Watered when soil moisture decreased to 1 atm.	—	—	—	—	2·3333	0·5378	1·7955	1·7580	0·3558	1·4022	1·3550	0·3313	1·0237	1·3486	0·3346	1·0140	1·0780	0·2815	0·7965	1·0229	0·2847	0·7382	1·0075	0·2783	0·7292	0·9161	0·3441	0·5720	0·8980	0·3433	0·5547	
3. Watered when soil moisture decreased to 8 atm.	—	—	—	—	2·4818	0·4744	2·0074	1·8430	0·3890	1·4540	1·5272	0·3548	1·1724	1·4567	0·3554	1·1013	1·2207	0·3136	0·9070	1·1935	0·3128	0·8807	1·1501	0·3178	0·8323	1·1719	0·4191	0·7528	1·1259	0·4309	0·6950	
4. Watered when soil moisture decreased to 15 atm.	—	—	—	—	2·6152	0·5669	2·1483	2·1572	0·4330	1·7243	1·8996	0·3848	1·5148	1·5939	0·3853	1·2086	1·4957	0·3833	1·1124	1·5060	0·3947	1·1113	1·4886	0·4021	1·0865	1·4674	0·4935	0·9739	1·3817	0·4880	0·8937	

progressively decrease with decreasing moisture status. The differences between field capacity and all other treatments were significant but the difference between 1 and 8 atm was not significant. Marginal means for varieties indicate that the var. RSK had the longest ear and this was followed by var. T55 and Hybrid in decreasing order. The marginal means for moisture regime for the character of grain yield indicated that yield decreased with decreasing moisture regime and all the differences between the treatments were significant except that between field capacity and 1 atm. Marginal means for varieties suggested that var. Hybrid had significantly higher yield than var. RSK and T55 but the difference between the latter two was not significant. The magnitude of effects caused by moisture regime was, however, different in the three varieties. Results indicate that in var. Hybrid, which showed the highest yield, decrease in soil water regime brought about a larger depression in yield in comparison with the other two varieties. Although under 15 atm regime yield was more or less the same in all the three varieties, var. Hybrid had a significantly higher yield than the other two under 8 atm. At field capacity and at 1 atm regime var. Hybrid, RSK and T55 had a decreasing order in yield and the differences in every case was significant.

Nitrogen status of shoot tissue—The total, soluble and protein nitrogen contents (as per cent) in the shoot tissue of the three varieties at their successive stages of growth under different moisture regimes have been presented in Table II. The results suggest the following facts:

(*a*) Irrespective of the variety, concentration of total nitrogen, at all stages of growth, remained much higher in plants grown under lower moisture regime in comparison with those raised under higher soil moisture conditions. As a matter of fact, in most cases, a progressive increase in total nitrogen content could be found with decreasing moisture regime.

(*b*) In general, nitrogen content decreased with the age of the plant.

(*c*) Soluble nitrogen content also showed a small increase under lower moisture regime and its level declined with the age of the plant.

(*d*) Protein nitrogen showed a marked increase at all stages of growth under lower moisture regime in all the three varieties. A decline in concentration was observed with increasing age of plants.

The dry matter production per plant at different growth stages has been presented in Fig. 2. It may be observed that dry matter decreased with decline in moisture regime in all cases, the minimum being in plants watered at 15 atm tension. However, it is apparent that var. RSK had the highest dry matter production followed by var. Hybrid and T55. This trend was reflected also in the data obtained at the harvest. But, it seems that the differences between the range of field capacity and 8 atm were relatively less (except perhaps in var. RSK) but at 15 atm the curve diverged to maintain a very low value as compared to others. This aspect in the context of nitrogen

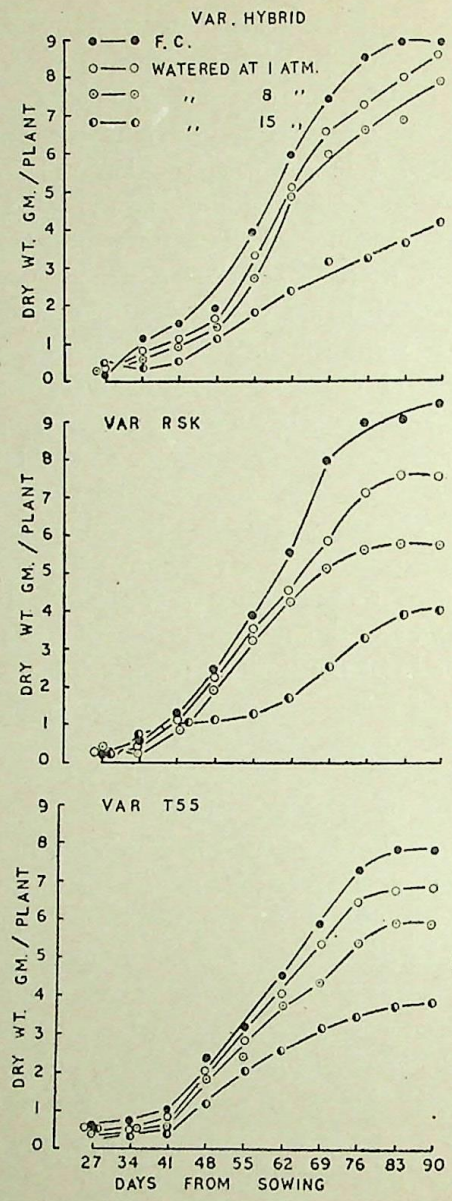

FIG. 2. Dry matter production in var. Hybrid, RSK and T55 on different days from sowing.

status is being discussed because on the basis of these values nitrogen content per plant was determined (Fig. 3). It is obvious from these graphs that nitrogen content per plant decreases markedly under lower soil moisture conditions although the concentration per unit tissue weight may be high. However, it is interesting to note that in all the varieties total, soluble and

protein nitrogen per plant were more or less comparable with each other between the range of field capacity and 8 atm regime. Between this range there was no consistent relationship between decreasing moisture regime and the total, soluble and protein nitrogen contents per plant. However, at 15 atm regime there was a marked decrease in the levels of nitrogen fractions in comparison with other moisture levels. This decrease was very much magnified in the case of total and protein nitrogen contents per plant while in case of soluble nitrogen the difference was small, and often, as in the case of var. RSK, not conspicuous.

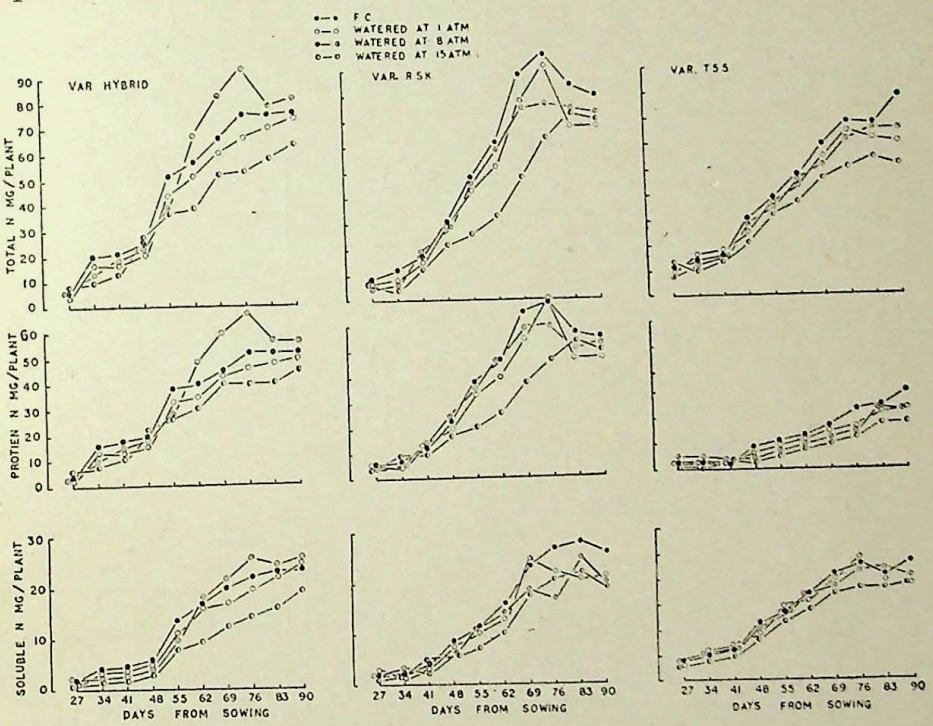

FIG. 3. Absolute quantities of total, soluble and protein nitrogen contents per plant on different days from sowing.

Rate of nitrogen uptake—The rate of nitrogen uptake under different moisture regime has been presented in Fig. 4. In a generalized way it may be said that in all the three varieties there are two distinct peak periods of nitrogen uptake, the latter being bigger than the former. In var. Hybrid, the first peak falls between 27 and 34 days, a period during which tiller number in the plant increases at a very fast rate. The second larger peak in this variety came at the time when the increase in the height of plants was about the fastest. The emergence of ear took place at a time when the uptake rate had considerably declined. However, the transformation of the shoot apex from the vegetative to reproductive phase must have started much

earlier and thus it may be possible that the second peak is associated both with the grand period of growth, as well as with the nitrogen demands at the very early stages of the reproductive phase. Whatever the case may be, it seems that the curves obtained for field capacity 1 and 8 atm were more or less identical in nature, whereas, at 15 atm regime, the uptake rates declined and both the peaks shifted possibly due to delay in flowering. In var. RSK the rates increased slowly to reach the first peak between 45 and 55 days and the maximum tillering was noted about the same time. The second larger peak followed immediately between 62 and 69 days. About this time the peak of growth in height was attained which was followed immediately by ear emergence. Under 15 atm regime decline in rates was associated with shifting of peaks but the ear emergence in this case also was found

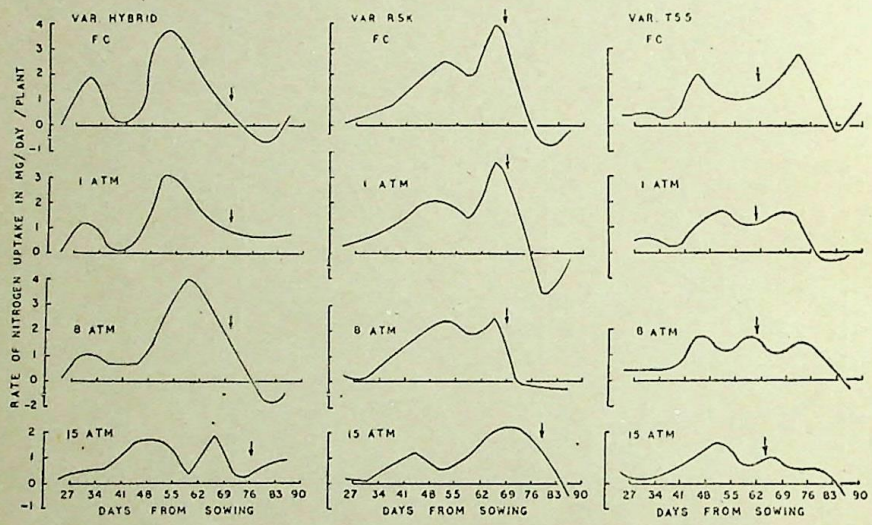

FIG. 4. Rate of nitrogen uptake in var. Hybrid, RSK and T55 under different soil moisture regime. Arrow indicates the stage of ear emergence.

just after the second peak. In var. T55, the situation was slightly different. Although the first peak was observed at a time when the tillering was maximum, it was followed by a rather long period of low rate (particularly under field capacity). Although, during this period, dry matter kept on increasing, the demand for nitrogen was apparently less, maybe due to lesser growth in this variety. But it is interesting to note that ear emergence took place just before the second peak which possibly suggests a greater demand for nitrogen in this variety during the post-earing phase. It seems further that this demand is not related with the growth as such, since the rate of extension growth virtually came to the very minimum during that period. It may be observed again that, in var. T55, the uptake rate was relatively less in comparison with other varieties, but as in other cases maximum decline in

rate was observed under 15 atm. The occurrence of third peak in between the first and second peaks, under 8 atm regime, could possibly be due to sample variations.

Nitrogen contents in grains—The results of total nitrogen contents of the grains have been presented in Table III. It may be found that the concentrations of nitrogen in the grains of all the three varieties tended to increase progressively with the lowering of the moisture regime. It seems that the

TABLE III

Nitrogen contents in the grains of var. Hybrid, RSK and T55 grown under different moisture regime

Variety	Total N (%) in grains under different moisture regime			
	F.C.	1 atm	8 atm	15 atm
Hybrid	1·44	2·31	2·79	3·54
RSK	2·33	3·52	2·50	3·49
T55	2·09	2·33	2·59	2·71

grain yield may be poor under low soil moisture conditions but the grains whichever were formed contained much higher nitrogen in comparison with those raised under higher moisture regime. It may be observed again that under 15 atm, var. Hybrid had the maximum concentration of total nitrogen in grains in comparison with other varieties while under field capacity the concentration was the least.

DISCUSSION

The results on plant performance indicate that the vegetative growth as such was maximum in var. RSK while the other varieties were comparable to each other. However, grain yield was highest in var. Hybrid but var. RSK and T55 had more or less similar yields. The greater yield in var. Hybrid could not be attributed to the length of ear (var. RSK having the longest followed by var. T55) but this could be due to a larger number of nodal ears and effective tillers and to better grain formation in the ears. Unfortunately, the number of ear-bearing tillers and nodal ears has not been recorded in this study.

The effects of moisture regime indicated that vegetative growth (as found in the final stage) was affected most adversely under 15 atm regime. The adverse effects on different vegetative characters between the range of field capacity and 8 atm regime were of different magnitudes. However, a decreasing trend with the decreasing soil moisture status was noted almost in all cases. The impact of moisture status on growth in height and leaf number was relatively less during the early stages except in the plants under 15 atm

regime which showed marked inhibition from the initial stages. But towards the termination of the vegetative period moisture variations brought about the expected differences. It seems, therefore, that the sensitivity to moisture variation increases towards the advanced stages of growth. A similar conclusion was derived in our earlier studies (Lahiri and Kharabanda 1965, Lahiri and Kumar 1966). However, soil moisture regime affected the tiller number at a relatively early stage, but this effect was magnified at the maximum tillering stage. These differences in tiller number possibly influenced the dry matter production.

Under 15 atm tension, it was found that var. T55 had a relatively higher growth in height in comparison with the other two varieties and it also showed early ear emergence. But the yield was highest in var. Hybrid. Although at 15 atm regime the yields of all the three varieties were comparable, var. Hybrid displayed a superiority over others between the range of field capacity and 8 atm.

The basic issue in this context is the extent of involvement of tissue nitrogen status in the foregoing growth and yield modifications. Ample evidences have been furnished to show that decrease in moisture regime tends to increase the concentration of nitrogen in the tissue. It seems that, when growth of plant is limited by soil moisture supply, nitrogen accumulates in the plant because the rate of entry is approximately maintained in conjunction with a decreased rate of utilization in growth process. Even under extreme conditions (as under 15 atm regime), where a substantial decrease in uptake has been observed, poor growth and dry matter production lead to manifold increase in per cent nitrogen in the tissue. In other words, under favourable soil moisture conditions, increase in tissue weight brings about a dilution and thus nitrogen content per unit tissue weight decreases. Therefore, it is obvious that the observed changes in growth and yield were not mediated by limitations of the tissue nitrogen level.

Such increases in total nitrogen concentrations have also been observed even in short duration experiments (Lahiri and Singh 1968). It was also found that water shortage leads to an impediment in protein synthesis and protein degradation, if any, occurs only when the plants are wilted. It was observed that water-deprived plants synthesized protein at a very fast rate when they were irrigated. These facts help us to explain the increased accumulation of protein nitrogen in plants raised under low moisture regime. Under the present experimental conditions plants were watered to field capacity when the soil moisture decreased to the desired tension. It is possible that large synthesis of protein during the 'wet phase' and its addition to the existent pool brought about an over-all increase in the protein reserve, although synthesis may be low during the 'dry phase' and some of it may be degraded during wilting under 15 atm regime. The increasing protein content

under decreasing moisture regime leads us to speculate that protein turnover during 'wet phase' was increasingly more under increasing tension. Less use of protein for growth processes in plants under low moisture regime was an added advantage in such accumulation. This may be the cause for the slight increase in soluble nitrogen. The decrease in concentration of nitrogen in the aerial parts with increase in age has also been observed by Myttenaere and Ringoet (1961) in rice and by Patel *et al.* (1958) in pearl millet.

However, it has been stressed by Stenlid (1958) that the absolute quantities of minerals per plant can help in the proper understanding of the physiological processes. The absolute quantities of total protein and soluble nitrogen per plant indicate that they were comparable with each other between the range of field capacity and 8 atm regime. But the curves obtained for 15 atm regime show a marked diversion to follow a low course. The differences for soluble nitrogen were relatively less because of the low quantity. These suggest that only at 15 atm regime there was a substantial decrease in the over-all nitrogen status of the plant. In certain cases a decrease (or loss) in the nitrogen content per plant has been observed in the advanced stages of growth. This could happen due to a number of causes like secretion and gaseous losses, translocation to other parts, abscission of leaves (Stenlid 1958), etc.

It was earlier believed (Hasselbring 1914) that 'the absorption of salts by roots is independent of the absorption of water and the transpiration stream does not exert an accelerating effect on the entrance of salts'. This view which was held as late as the middle thirties has undergone a change in the subsequent years and now it is more or less established (Wright 1939, Broyer and Hoagland 1943, Hylmö 1953) that increase in water uptake may increase in the rate of ion uptake and a relation exists between water and ion uptake. Jensen (1962) observed that water and nitrate absorption follow uniform rates and an increase in suction increases the rate of uptake of both water and nitrate. If we assume that water is equally available to plants between the range of field capacity and permanent wilting percentage (Veihmeyer and Hendrickson 1950), differences in nitrogen uptake rates may not be expected except under 15 atm regime, as it has been observed here. It was also demonstrated in an earlier study (Lahiri and Kharabanda 1966) that transpiration rate does not alter due to variations in soil moisture over a wide range and it decreases only when soil water decreases close to the permanent wilting percentage.

However, the rate of uptake has been found to change at different stages of growth. The double peak nature of the course of nitrogen uptake remains more or less unchanged under different moisture regimes and alterations in the position of peaks have been found under the 15 atm regime, presumably

due to changes in growth behaviour and time of earing. It may be mentioned in this context that Tanaka *et al.* (1959) also observed two distinct peak periods in nitrogen uptake of rice varieties. However, in the case of rice ear emergence is immediately followed by the second peak and therefore the second peak is usually related with the nitrogen demands at earing. A similar situation in var. T55 has been observed here. But in var. Hybrid and RSK the second peak, which is relatively larger than the first, occurs just prior to earing. It may be possible that in these varieties demand for nitrogen is higher during the early reproductive phase of the shoot apex and partially it may also meet the demands for growth. Whatever the case may be it seems that the second peak is larger than the first which is related with the tillering process.

Applications of high doses of nitrogenous fertilizers are not favoured by Agronomists under arid conditions. Under unfavourable conditions it leads to the so-called 'burning' of the crop. Our studies indicate that decrease of soil water close to permanent wilting only impedes the intake of nitrogen. The adverse soil moisture conditions, however, affect the photosynthetic efficiency and dry matter production. Under high dose of fertilizer increased vegetative growth is expected to favour greater soil water exploitation. Consequently, if plants wilt, it will automatically increase the ammonia nitrogen in the tissue (Lahiri and Singh 1968) which being toxic may cause the burning. Many other diverse causes may also be related with this process.

It has been demonstrated that the yield decreases under lower moisture regime but the concentration of nitrogen in grains whichever were formed contained much higher nitrogen. This phenomenon has also been observed in wheat by Asana and Sahay (1965) and they explained that this is due to lower accumulation of dry matter in grains. It has been suggested by Petinov and Pavlov (1955) that flow of nitrogen into the grains is less subjected to unfavourable moisture conditions than the flow of carbohydrates.

In conclusion it may be said that the information provided in this investigation presents only a part of the complex influence of soil moisture on plants. It is possible again that the processes of accumulation of other nutrients are altogether different from those outlined here for nitrogen.

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EFFECT OF RADIOACTIVE PHOSPHORUS AND SULPHUR ON INFECTIVITY OF TOBACCO MOSAIC VIRUS IN TISSUE-CULTURE

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Studies conducted to see effect of radioactive phosphorus and sulphur on a strain of TMV (CPO Strain) in tissue culture showed that there is greater uptake of radioisotopes by virus-affected tissues as compared to healthy ones, when grown on medium containing the radioisotope at an activity of 15 microcuries per ml. Quantitative data on uptake showed that it is more in differentiated tissue than callus only. Radioactive phosphorus at this dose has no effect on the infectivity while radioactive sulphur at the same dose inhibited the infectivity by about 90 per cent.

Introduction

Inactivation of plant viruses by radiations has been reported by several workers and much work has been done by *in vitro* studies as well as on the host plant (Bawden and Pirie 1937; Pollard and Dimond 1956; Raychaudhuri 1963; Nariani and Paliwal 1963). Although radioactive isotopes have been extensively employed in the studies on absorption and translocation of various metabolites in different plant parts, their use in the inhibition of plant viruses in the host or tissue culture has not been much investigated. Schlegel *et al.* (1953) reported inhibitory effects of P^{32} in certain plant viruses like TMV. Chatrath and Raychaudhuri (1967) who reported greater accumulation of P^{32} in mosaic affected sunnhemp plants suggested its incorporation into virus particle and possibility of therapy of plant viruses by this method.

The present investigations were undertaken to study the effect of radioisotopes on a strain of TMV (CPO strain) in tissue culture by incorporating them into the medium.

Materials and Methods

'CPO Strain of TMV' maintained in normal leaf callus cultures of *Nicotiana tabacum* var. *xanthii* was employed for the present studies. These cultures are maintained on Murashige and Skoog's medium (1962).

Carrier-free radioactive phosphorus (P^{32}) and sulphur (S^{35}) were obtained from Atomic Energy Establishment, Trombay, in the form of H_3PO_4 and H_2SO_4, respectively. These were neutralized with $N/10$ NaOH and were

added to the medium to get the required activity. This was sterilized at 15 lb pressure for 20 minutes. Tissues approximately of the same size were transplanted into these tubes which were kept under continuous daylight (fluorescent tubes) at 25–28 °C for growth. After a growth period of three weeks, the tissues were washed thoroughly in water to remove any traces of medium sticking to it. Half of the tissues from each plant was used for infectivity tests while the other half was dried at 70–80 °C for 72 hours, powdered and weighed aliquots were deposited in a thin layer on aluminium planchets (Phillips). The radioactivity of samples was measured by Geiger Mueller Counter (Phillips Universal Scaler Model PW 4032) using GM tube Mica and window type. This was recorded in terms of disintegration per minute per milligram based on a total of at least 10,000 counts. At least two samples were counted for each treatment and average values recorded. Planchets containing dried tissue for counting were supported by two empty planchets to increase the back-scattering so that samples with low level of activity could be measured efficiently.

For estimating the infectivity, callus cultures were weighed and crushed for preparing the standard extract which was diluted ten times with distilled water. For suitable controls, tissues of the same growth age and approximately of the same size, grown on the medium without any addition of radioactive material, were taken and crushed for preparing the inoculum as above. Bioassaying of viral infectivity was done by inoculating the local lesion hosts, i.e. *Chenopodium amaranticolor*. Different treatments (P^{32} and S^{35}) including the control were distributed on different leaf positions according to Latin square design. Local lesions were counted after five days and infectivity expressed in terms of local lesions per leaf.

Results

In both the experiments with P^{32} and S^{35}, radioactive solutions were added to the medium to get an activity of 15 microcuries per ml (total 10 ml medium in each tube).

Table I

Relative activity of samples of healthy and diseased tissues of Nicotiana tabacum *var.* xanthii *grown on medium containing* P^{32} *and infectivity of irradiated and uniradiated tissues*

Type of Tissue	Disintegration per min. per mg		Infectivity (average number of lesions per leaf)	
	Healthy	Diseased	Irradiated	Checks
Undifferentiated callus	1537·6	3289·2	22·4	19·02
Differentiated callus	5638·0	9150·4	40·9	39·87

The results on radioactivity and infectivity of samples grown on medium containing P^{32} or S^{35} are given in Tables I and II.

It is observed (Table I) that there is greater uptake in the differentiated tissue as compared to undifferentiated callus. In both callus and differentiated virus infected tissues, radioactivity was found to be much more than healthy tissues. Though infectivity in the differentiated tissue is more as compared to callus, there was no difference between irradiated and uniradiated tissues. In the case of senescent tissues, there was no difference in activity or infectivity between healthy and diseased and irradiated and uniradiated tissues, respectively.

TABLE II

Relative activity of samples of healthy and diseased tissues of Nicotiana tabacum *var.* xanthii *grown on medium containing* S^{35} *and infectivity of irradiated and uniradiated tissues*

Type of tissue	Disintegration per minute per mg		Infectivity (average number of lesions per leaf)	
	Healthy	Diseased	Irradiated	Checks
Undifferentiated callus	1580·8	1364·4	135·5	92·0
Differentiated callus	2064·8	5585·6	9·0	104·5

It would be observed that uptake of S^{35} is much more in virus-affected differentiated tissues as compared to healthy tissue and at this level of activity infectivity of virus is greatly reduced in tissues grown on medium containing S^{35} as compared to uniradiated controls.

In both experiments with P^{32} or S^{35}, no adverse effect on the growth of tissue was observed at these dosages.

DISCUSSION

The results of these experiments indicate that uptake of radioactive phosphorus and sulphur is more in diseased differentiated tissues as compared to healthy ones. Activity was also found to be more in differentiated tissues than in callus only. Higher uptake of P^{32} and greater infectivity in differentiated tissues as compared to loose mass of callus growth suggest that phosphorus which is an important constituent of nucleic acid and on which infectivity of virus depends is incorporated into the virus. Stanley (1942) also reported incorporation of P^{32} into virus of infected tobacco plants grown on nutrient solution containing P^{32}. However, incorporation of P^{32} into the medium at this dosage has failed to inhibit infectivity of virus-affected tissue.

In case of S^{35}, there is no difference in the activity or infectivity of healthy and diseased callus cultures. However, infectivity of irradiated differentiated

tissue was much reduced as compared to uniradiated checks, indicating inhibition of viral infectivity. Failure of S^{35} to inhibit viral infectivity in callus growth may be due to the possibility that virus multiplication in callus might have been completed before sufficient S^{35} accumulated in tissue to cause any reduction. Also infected callus contains only one quarter as much S^{35} per mg of tissue as the corresponding differentiated ones.

As the dose of S^{35} used in these investigations does not have any adverse effect on tissues, it suggests the possibility of application of radioisotopes in therapy of virus diseases and utilizing this method for obtaining virus-free tissues.

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